I dedicate this thesis to my Parents Keith & Patricia Hancock and

Grandmother Barbara Fletcher for their patience, support,

encouragement and continued belief in me.

You were always there for me even when I was not there for you.

For Mum

Tiger tiger burning bright
in the forest of the night,
what immortal hand or eye,
could frame thy fearful symmetry?
When the stars threw down their spears
And watered heaven with their tears,
Did He smile his work to see?
Did He who made the lamb make thee?

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Twas brillig, and the slithy toves
Did gyre and gimble in the wabe;
All mimsy were the borogroves
And the mome raths outgrabe".
One, two! One, two! And through and through
The vorpal blade went snicker-snack!
He left it dead, and with its head
He went galumphing back".

Jabberwocky

Lewis Carol

"Water, water everywhere, Nor any drop to drink"

The Rime of the Ancient Mariner
Samuel Taylor Coleridge

Aims

The aims of this research were 2-fold.

- Firstly, to monitor the fate/sorption of phenols on different soils over extended time periods in an attempt to correlate sorption to the properties of the soil matrix. This would provide information which could be used for the prediction of the sorption of phenols on unknown matrices and hence to their fate in the environment.

- Secondly, to develop an optimised method for the extraction and quantitation of pollutants in liquid and solid matrices using the recently developed technique of solid phase microextraction. This work was carried out in an attempt to provide a viable 'environmentally friendly' alternative to traditional sample preparation techniques, such as, shake flask, Soxhlet/solvent reflux (for extracting analytes from solids) and liquid-liquid extraction (for extracting analytes from liquids).

Abstract

Different sample preparation methodologies were evaluated for the determination of pollutants in different matrices. The methods investigated were chosen on the merits of decreased sample preparation time and low toxic solvent consumption with the aim to provide viable alternatives to more laborious methods, such as, Soxhlet extraction. Techniques were developed to extract and quantify organic pollutants from contaminated soils and water.

The results from shake flask extraction of aged phenolic contaminated soils were used in attempt to relate sorption to both the soil, and pollutant properties. This was to help gain an understanding of the transport and fate of phenols in different environmental situations. The partitioning (sorption/desorption) of radiolabelled phenols between aqueous solution and soil was investigated using a modified shake flask technique. This provides additional information which can be related to soil characteristics, hence pertaining to the fate mechanisms involved for phenols in the environment. The results from these investigations suggest that more than one factor contributes to the sorption of phenols in soils.

Solid phase microextraction (SPME) was initially evaluated as a semi-qualitative tool for the extraction of analytes from several different matrices. The use of SPME was further applied to full qualification and quantification of extracted chlorobenzenes and PAH's from soils and water samples. Shake flask, accelerated solvent extraction (ASE) and Soxhlet were also used, to allow comparison with SPME. The work involving SPME showed that with further method modification this technique may be used for a diverse range of future applications for the extraction and quantification of analytes from liquid or solid matrices.

The methods used to quantify the levels of extracted pollutants in these experiments were high performance liquid chromatography with ultraviolet detection (HPLC-UV), liquid scintillation spectrometry (LSS), gas chromatography with electron capture detection (GC-ECD) and mass spectral detection (GC-MS).

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Chapter 1.0

Environmental matrices and pollution

1.1 Introduction

Due to the expansion of many polluting industries the presence of synthetic organic compounds in the environment is growing. Anthropogenic sources include agricultural and silvicultural application, accidental spills and surface/subsurface waste disposal [1-3].

Natural and anthropogenic substances share the second most common organic characteristic in the biosphere, the aromatic nucleus (benzene ring) [4]. In the top 50 chemicals manufactured annually in the United States of America (US), aromatics constitute c.a. 91 billion kg [5]. With these quantities in mind it is not surprising that growing concern surrounds the environmental impact caused by accidental spillages. Protection of the environment has become a pressing issue world-wide and governments of all developed countries have responded to this global threat by passing and enforcing regulations for environmental protection [6]. This awareness in recent years has fuelled research into the effects that pollutants pose to the environment we have to live in.

Although at present a great deal is understood about the transport, fate, toxicity, bioacumulation and degradation of pollutants and general trends can often be concluded, there are many differences in the methods used to measure or predict these effects. Standard calibration curves of concentration v's response are used to find the unknown concentration of an analyte. Similarly, physiochemical parameters are often used to develop models which can predict certain behavioural aspects of a known chemical under different conditions. Such models are developed from correlations between experimental observations and the physiochemical characteristics shown by groups of chemicals.

Prediction of the environmental fate of organic compounds depends in part on the quality of the physiochemical data available. The results from physiochemical determinations are often very different, and this is one source that can lead to error in the formation of mathematical models for use in predicting the environmental impact of compounds. For example, the water solubility of phenol according to ref. [7] is 93 g/l, ref. [8] 87 g/l and ref. [9] is 59.4 g/l. Another example is that of Henry's constants (H) which can be used to correlate the concentration of an analyte in the vapour phase with experimental data [10, 11]. Table 1.1 highlights the variation in the literature values for PAH's (All values are taken from reference 10).

Table 1.1

Literature values for Henry's constants

	Henry's constants (H) ($Pa.m^3.mol^{-1}$)			
	H calculated Ps/Cs	H experimental gas stripping	H experimental gas stripping	H experimental wetted-wall column
Naphthalene	42.98	44.6	48.94, 56, 42.5	74.4
1-methylnaphthalene	44.87	24.3	26.3	62
Fluorene	7.89	9.75	11.86	6.45
Phenanthrene	3.24	3.61	3.98, 5.55, 4.68	2.38
Anthracene	4	7.66	6.59, 7.4, 3.3, 4.94	1.96
Fluoranthene	0.95		0.65	
Pyrene	0.92	1.21	1.89, 1.1	

From table 1.1, Henry's constants which are calculated from vapour pressure (Ps) and solubility (Cs) data (Ps/Cs) are significantly different to values obtained experimentally. Experimental values also differ depending on the technique employed.

Such differences will undoubtedly affect the accuracy of any prediction model and one should be aware of this when choosing and applying physiochemical data in this way from literature values.

The different offices of the US 'Environmental Protection Agency' ('EPA') are centered around types of sample media: water, solid waste, air and pesticides. Each office has set up its own research programs and developed its own analytical methodologies and as a result there is a proliferation of methods.

For example, for the determination of chlorobenzene there are 13 GC methods available [12]. As the bulk of environmental analysis in the US is carried out in private laboratories, this has lead to confusion as to which is the proper method to use with a specific sample [12]. When relevant, the lack of standardisation of certain aspects of methods in the literature will be highlighted in later chapters.

1.2 Reference materials

Long and complex experimental procedures are more difficult to successfully repeat due to the build up of errors associated with each step. To address this problem, certified or standard reference materials (CRM's or SRM's) are often used to compare results from a single method employed by different laboratories.

With an estimation that in 1988, 250 million chemical analyses were performed in the US each day, the reliability of these results is economically important with a cost of 5 billion dollars spent on repeating poor measurements [13]. The implications of different

laboratories obtaining different results for the same analysis can be potentially very damaging. In a typical example, when 25 laboratories analysed the total cyanide content in a contaminated land sample supplied by the Laboratory of the Government Chemist (LGC), the results ranged from 533 to 6983 mg/kg [14].

The importance of CRM's/SRM's is to establish the traceability of measurement results and ensure the accuracy and reliability of the results of chemical analyses. An 'accurate measurement' as defined in the International Vocabulary of Basic and General Terms in Metrology (VIM) [15] is one where the measurement result is 'very close' to the 'true value' of the measurand. Such accuracy is required in order to control the quality of products and services provided by industry and to monitor the environment whilst at the same time constituting a basis of comparability of analytical data. By definition, a reference material is a stable material or a substance whose chemical or physical properties are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [16].

Environmental matrices exist as CRM's/SRM's for the measurement of many important pollutants, and as stated by the European Commission, Community Bureau of Reference (BCR) guidelines the production of specific reference materials is fuelled by their demand, highlighting the increased awareness towards environmental pollution. As an example of their widespread and often novel use, reference materials exist for polyaromatic hydrocarbons (PAH's) and phenols in shale oil (SRM 1580), pesticides and polychlorinated biphenyl's (PCB's) in cod liver oil (SRM 1588), PCB's in human serum (SRM 1589) and PAH's in diesel particulate matter (SRM 1650) and with over 200

suppliers world-wide their importance is well established [16, 17]. However, laboratory handling exposes reference materials to temperatures higher than storage conditions and once opened CRM's may absorb water which may affect their stability. Recently the stability of certain compounds contained in CRM's has come into question by some authors [18, 19]. Very low recoveries of 2-methylphenol (17.7 %) [18, 19] naphthalene and 1,2,4-trichlorobenzene [19] have been found on a soil supplied by Environmental Resource Associates (ERA soil Lot. nos. 329 & 323). 2-methylphenol was also found to have low recoveries from laboratory spiked soils [20]. There are also problems with the stability of complex biological matrices. For example at storage temperatures of -30 °C vitamin C is still unstable in brussels sprouts over a 12 month period [21].

1.3 Environmental matrices

Sorption and degradation are the main processes which attenuate the transport, bioavailability and hence toxicity of organic compounds in soil and water systems. The field of environmental analysis is largely centered around measuring the partitioning of different chemicals between different matrices in order to form models able to predict the fate of these compounds in the environment.

When a compound or mixture of compounds are released into the environment (if we neglect degradation and metabolisation) they will come to equilibrium between many different matrices depending on their affinities for each phase. The ratio of the affinity for a chemical between phases is called the partition coefficient and is generally only measured under laboratory conditions for 2-phase systems such as (air/water, air/leaves, air/soil, water/soil, water/roots and water/lipids) due to the complexity involved. At a

molecular level, this affinity will largely depend on the specific sorption interactions between different functional groups of the analyte and the matrix.

In 'soil science', sorption is generally described as a partitioning process between aqueous solution and soils or sediments [22-26]. While chemical processes may result in partial breakdown of organic compounds [27] and possible incorporation into humic substances [28-33], microbial degradation is said to be the only process that can result in the complete mineralization of these compounds [34-41]. The extent of sorption is related to various soil properties and also to the physical and chemical properties of the target analyte. The major factors determining the extent of sorption and degradation of organic compounds under field conditions include :

1.3.1 Properties of the soil system

- Chemical properties [42-46]: moisture, water holding capacity, pH, cation exchange capacity (CEC), % organic matter or carbon and clay mineral type e.g. kaolinite '$Al_4Si_4O_{10}(OH)_8$' and montmorillonite '$Na(Al,Mg)_4Si_8O_{20}(OH)_8$'.

- Structure: bulk density, surface area, pore structure, heterogeneity.

Colour of soil is also important in the top layers exposed to sunlight. Darker soils will heat up quickly promoting water loss and increased sorption of organic compounds as well as photo oxidation.

1.3.2 Nature of chemical applied

- Physical properties [47-48]: size and shape.

- Chemical properties [47-48]: solubility, vapour pressure, acid dissociation constant (pKa) and hydrophobicity as measured by the octanol/water partition coefficient (Kow).

1.3.3 Field conditions

- Climate (seasonal, daily), rainfall, temperature, solution pH, ionic strength.

Knowledge gained from this information concerning the interactions of different chemicals with different matrices also gives the analyst a good starting point for developing efficient methods for the extraction and measurement of different compounds from different matrices.

1.4 Soil organic matter

Soil organic matter is often linked with sorption of chemicals in the environment [42-46]. Soil organic matter is a complex mixture of macromolecules of fulvic acids, humic acids and partially decomposed cellular substances, such as lignin, which is closely linked with cellulose in the woody tissues of plants. Whilst cellulose is readily decomposed by micro-organisms, the methods for lignin degradation are not well known at present [49] but it has been suggested as a precursor to humic substances [50-51].

Lignin is an aromatic 'polymer' made up of randomly linked phenylpropanoid units rich in methoxyl groups. Lignins lack of carboxylic substituents makes it much less hydrophilic than humic and fulvic acids. Conversely fulvic acids have a larger proportion of carboxylic groups than lignin and humic acids and are therefore more polar in nature.

Due to the physical and chemical nature of these biopolymers, they possess different sorptive properties which will have important implications in soil systems. For example, the higher solubility of fulvic acids to humic acids will aid in transport of fulvic acid bound organic compounds through the soil profile. Water soluble 'humic materials' may form micelles that can partition organic compounds in their hydrophobic interiors thus increasing their transport and ultimate fate in the geological profile [52]. Also one would expect lignaceous polymers to have a greater affinity to sorb nonpolar organic compounds whilst fulvic acids will have a higher affinity for polar organic compounds.

As a mixture of natural polymers, soil organic matter has been found to adhere to mineral surfaces and behave like a partition medium for organic chemicals [53]. Applications have recently been developed which mirror this finding. These include the use of modified porous silica particles with anion-exchanger functionalities for the removal of humic acids from river water [54] and the coating of liquid (or solid) polymers on silica fibres for use in solid phase microextraction (see part B chapter 7.0).

It is reasonable to expect organic matrices in natural systems that have varying origins, degrees of humification (aromaticity), and degrees of association with inorganic matrices to exhibit dissimilarities in their ability to sorb organic compounds. Indeed, it has been shown that sorption of organic compounds varies with the geological age of

organic matter [44,55], from partitioning in amorphous 'soft' humic materials to sorption in increasingly condensed microcrystaline structures formed as diagenesis takes place. In contradiction to a generally accepted view of universal partitioning in humic acid, Mingelgrin and Gerstl [26] proposed that "the physico-chemical nature of the organic matter varies from soil to soil and can't therefore be treated as a well defined organophilic phase". Such complexity reflects the lack of reliable information to date concerning the tertiary and quaternary structures of soil humic and fulvic acids [56]. Fulvic and humic acids range from yellow to dark brown and have structureless broad absorption spectrums which extend from the near UV and visible region to the far-red region with decreasing absorption [57]. Due to the acidity and polarity of humic and fulvic acids they are soluble in water and as a result they are eluted close to the solvent front in reversed phase HPLC. Their broad absorption can be a source of matrix interference at many wavelengths for compounds extracted and analysed from humic rich environments [58-59].

1.5 Clay minerals

Clay minerals are another important constituent of the soil matrix which vary from soil to soil and have an effect on the sorption of native or anthropogenic organic chemicals. As the surface charge of individual mineral species influences the extent of interaction of dissolved organic matter one would expect this to be the same case with smaller organic compounds. The pH of the zero point charge (ZPC) (or isoelectric point) is the pH where the mineral surface is neutral. The ZPC for some common minerals are given in table 1.2 [60].

Table 1.2

The zero point charge for some mineral surfaces

Mineral	Formula	ZPC
Kaolinite	$Al_4Si_4O_{10}(OH)_8$	3.3 - 5
Montmorillonite	$Na(Al,Mg)_4Si_8O_{20}(OH)_8$	2.5
Quartz	SiO_2	1.8 - 2.4
Ferrihydrite	$Fe_2O_3.\ nH_2O$	8.5 - 8.8
Goethite	α-FeOOH	7.8-8.3
Calcite	$CaCO_3$	9.5 - 11

Although most clay minerals (as with humic acids) have a 'net' negative charge, clay edge sites can be positively charged. The 'net' negative surface charge on clay minerals is due to isomorphous substitution (for example Al^{III} for Si^{IV}). Negatively charged mineral surfaces such as sand and Celite do not interact with humic acids or dissolved organic matter. For the same reason, phenols would not be expected to irreversibly interact with sand or Celite due to the electronegative oxygen of phenols, which would be repulsed by the mineral surface.

Conversely, minerals with positive sites such as goethite and calcite have strong sorption affinities for dissolved organic matter due to surface complexation with phenolic and carboxylic functional groups. This results in a high association of humic acids or dissolved organic matter with these minerals and leads to an increase in the sorption capacity of the mineral surface. One would expect these minerals to have the same effect for complexation of phenols,

1.6 Celite

Diatoms are one-celled golden brown algae which have an intricately structured amorphous siliceous skeleton. Diatomaceous earth consists mainly of accumulated shells or frustules which are the remains of fossilized diatoms [61]. Pure Diatomite or 'Celite' is white and essentially inert consisting primarily of silicon dioxide SiO_2 (87.9-90.8 % wt). Celite can contain between 3.54-4.06 % wt Al_2O_3 (corundum) and 1.4-1.54 % wt Fe_2O_3 (ferrihydrite) and also smaller quantities of CaO, Na_2O, P_2O_5, MgO, K_2O, and TiO_2. Celite has high water absorption and permeability due to a high surface area (1-6 m^2/g) resulting from the large network of pores through the silica cell wall with a network of smaller secondary and tertiary pores. Although Celite is said to be an inert matrix, it has been found to considerably retain organophosphates [62]. Other than this information little else has been found concerning the surface properties of Celite. Celite is structurally different but chemically equivalent to sand, so some comparison may be made in this respect. Assuming that Celite is mainly SiO_2 with a small percentage of positively charged minerals such as ferrihydrite we can estimate that the ZPC of Celite will be a little higher than that of sand or quartz. Therefore the surface charge of Celite should be predominately negative.

1.7 Environmental pollutants

Phenols, chlorobenzenes and PAH's were chosen for these studies as they are three groups of pollutants which are released in substantial quantities into the environment. They do not exist as single contaminants but as components of complex mixtures. In one example, chemicals from all three classes have been found in polymeric

building materials [63]. These are one source of indoor chemical emission that can cause poor air quality in buildings (termed "sick building syndrome") resulting in impaired health. Various types of vinyl floorings and carpet underlays have been shown to release phenol, cresols, ethylphenols (used in dye plasticizers and polymer preparations), naphthalene, methylnaphthalenes, as well as mono-, di- and tri-chlorobenzenes [63]. Chlorobenzenes and PAH's have also recently been found in the extracts of pine needles and mosses [64]. In this section the different implications that each group has towards the environment and health will be discussed.

1.7.1 Phenols

Phenol is one of the most widely used compounds in existence [37,65] and is listed as a priority pollutant by the US EPA [66]. 1.25 billion kg phenol was produced in 1979 [67]. Specific commercial products requiring phenols include explosives, paints, perfumes and fabricated plastics. Phenol is also a precursor in the synthesis of adhesives and is used as a preservative in many types of pharmaceutical and cosmetic products [67]. 1-naphthol and 2-naphthol are used as antioxidants (preservatives) in the rubber industry and in the preparation of dyes and medicinal organics [68, 32].

As a result of their widespread applications, phenols are released into the environment from several anthropogenic sources which include waste water from the petrochemical industry, coal gasification plants, resin manufacturers, dye synthesis, pulp and paper mills and aircraft maintenance. Phenol is present in high concentrations in these wastes and for example has been detected at levels of 800-2000 mg/l in plastic factory waste and 580-10000 mg/l in the weak ammonium liquor of a coking plant. In the pulp

and paper industry, the hydrolysis of lignin yields a wide variety of phenols including phenol, methyl phenols, ethyl phenols, xylenols, catechols, guaiacols, and syringols [69]. Phenols are also naturally present in surface and ground waters as degradation products of lignin as well as algal secretions [70]. In nature biological oxidation and coupling of phenols are key reactions that result in the formation of products such as lignins, melanins, tanins, alkaloids and antibiotics [7,71].

Phenols are a major group of chemicals from the wood-preserving process, and are quite effectively biodegraded during ground water transport and also occur as aerobic degradation products of non phenolics [72-73]. For example, pesticides such as Silvex™ degrade to trichlorophenol and dichlorocatechol [74], whilst 1-naphthol is a synthetic precursor and a degradation product of the insecticide Sevin [75]. 1-naphthol is also released into the environment as a component of the herbicide napropamide [76] and as the result of the oxidation of naphthalene by certain fungi and bacteria and is toxic to marine invertebrates [77]. 1-naphthol is also a well known degradation product of Carbaryl which is one of the most widely used industrial insecticides. In 1988 c.a. 11 million kg of Carbaryl was applied to crops in the US to eliminate chewing and sucking insects [78-79]. Carbaryl has low acute toxicity to mammals and does not persist in blood or accumulate in tissues of humans, and is metabolised to 1-naphthol which is excreted in the urine [79].

Treatment methods for the removal of phenols from waste waters have recently included anaerobic biodegradation via nitrate respiration and methanogenic fermentation [34-37,80-81] and chemical oxidation with ozone [82-83] whilst removal and recovery has been achieved using synthetic high surface area sorbent resins [84-86]. The

environmental fate of phenolics is ultimately determined by their ease of degradation and sorption interactions with soil or soluble humic compounds [87-92]. It is also known that metal oxide soil coatings can catalyse oxidative coupling of phenols to form polymerised products which can be incorporated into soil organic matter [71, 93].

River water bacteria have been shown to metabolise 1-naphthol to CO_2 or transform it into high molecular weight compounds [68] whilst filamentous fungi have been found to synthesise phenols and incorporate them into 'humic acid' type polymers [94, 32-33]. These are therefore an important source of phenol degradation as they grow frequently in wood and soil where phenolic structures are present [95]. Sjoblad et al. [78] established that a fungus can form an extracellular enzyme (assumed to be a phenol oxidase) which is active in polymerising 1-naphthol, phenol and p-methylphenol.

An extensive review on the health risks of phenol has been given by Babich and Davis [96]. Phenol is a normal constituent of animal and human tissues as it is formed in the human body due to high protein or meat diets. Moderate amounts can be detoxified in the body by conjugation with sulfonic and glucuronic acids and excreted in the urine as the glucuronide or sulfate ester. Foods found to contain phenol include smoked summer sausage (7 mg/kg) and chloroseptic lozenges (32.5 mg/lozenge) [96].

Although low levels of phenols have little adverse effect on health, high levels of phenols can reduce fertility and inhibit growth in biota and be toxic to other aquatic life [96,69] and pose a direct threat to human health, such as depression of central nervous system functions and kidney and retinal damage [97]. The lethal oral dose of phenol in mammals (LD_{50}) is 0.1-0.65 g/kg whilst the overt toxic response in invertebrates (LC_{50})

is given as 6.5-500 mg/l and vertebrates is 8-67.5 mg/l, depending on species. The oral toxicity of phenol leading to death in humans estimated from the total dose taken, is between 0.14-0.43 g/kg (probably due to suicide or medical misapplication) [96].

Babich and Davis [96] state that there has only been one incident attributable to environmental poisoning solely by phenol, before 1981. In 1974, 37,900 litres of 100 % phenol (as phenol is a solid, 100 % phenol presumably means phenol saturated solution) were spilled in southern Wisconsin after a train derailment. Following the accident, 1.13g/l were detected in well water. Inhabitants living near the site had an estimated intake of 10-240 mg phenol/day and developed mouth sores and skin rashes and experienced diarrhoea. In another case, poisoning of an aquatic ecosystem by 'phenols' occurred following the accidental release from a dike retaining industrial wastes from a phenol factory in Luxembourg. The stream contained more than 10 mg/l phenol and destroyed virtually all the aquatic flora and fauna [96].

Further concern surrounds the formation of chlorophenols from phenols by direct chlorination of water in disinfection plants. At part per billion (ppb) levels this class of phenolics have undesirable organoleptic properties in drinking water and fish. This process can also produce contaminants such as dibenzofurans and chlorinated dibenzodioxins (which can also be present as impurities in reagent grade phenols or as products from burning chlorophenol preserved wood [98]). Acute toxicity by monochlorophenols is similar to that of phenol with a convulsant effect, this effect diminishes with increased chlorine substitution. Minimum lethal dose of pentachlorophenol in humans has been estimated as 29 mg/kg [98] compared with 140-430 mg/kg for phenol [96]. The toxicity of chlorophenols in aquatic organisms attributed

to many factors including an uncoupling effect on oxidative phosphorylation and stimulation or inhibition of a number of enzyme systems. For example acute poisoning with pentachlorophenol (PCP) causes enzyme activity to increase in the citric acid cycle and respiratory chain. Therefore chlorophenols constitute another threat to the aquatic environment.

In soil systems humic acids or dissolved organic matter may be associated with $FeCl_3$ or $AlCl_3$ [99]. Ding and Xu [100] found that mixing powdered 2-naphthol and $FeCl_3.6H_2O$ or dissolving 2-naphthol in Fe^{3+} solutions, 1,1'-bi-2-naphthol formed in 95 % yield. A similar result was found by Bollag et al. [101] where 1-naphthol readily underwent oxidative coupling in the presence of $FeCl_3$ resulting in the formation of 2,2'-bi-1-naphthol and 4,4'-bi-1-naphthol. This in turn could be oxidised to an extended 'purple' quinone. The microbial oxidation of 1- and 2-naphthols to their dihydroxynaphthalene isomers has also been demonstrated by Bianchi et al. [102]. Wang et al. [103] succeeded in preparing model humic substances by reacting phenolic compounds with montmorillonite, illite and kaolinite mixed with quartz. A change of colour was observed in extracted clays aged with phenols from clear to reddish brown/pink and indicated that the addition of clays with good CEC to sand favours the accumulation of soil organic matter.

The formation of coloured quinones by oxidation of phenols in air (O_2) and coloured substances from various phenols by fungi (phenol oxidases) is well documented. The initial work of Bollag et al. [101] found that lignin decomposing fungi were able to oxidise 1-naphthol to water soluble products possessing an intense colour which turned the growth medium purple. Using radioactive naphthol in the growth medium they also

found that radioactivity in aqueous phase was associated with a relatively high molecular weight compound (> 200,000). Further studies by Sjoblad et al. [104] and Sjoblad and Bollag [105] isolated an extracellular phenol oxidase enzyme which catalysed the polymerisation of 1-naphthol and 2-naphthol to dimeric, trimeric, tetrameric and pentameric compounds. Phenol was also polymerised up to a tetramer and 4-methylphenol to a dimer as characterised by GC-MS. Polymers of higher molecular weight could be present but were probably too involatile to detect under the conditions used.

1.7.2 Chlorobenzenes

Chlorobenzenes are used as industrial solvents, pesticides, deodorants and chemical intermediates in the manufacture of pesticides , phenols and dyestuffs [106-108]. For example, 1,2,4-trichlorobenzene has wide usage including a solvent in chemical manufacturing, a carrier to apply dyes to polyester materials, a dielectric fluid in transformers, a component in lubricants as a heat transfer medium and as an ingredient in insecticides, herbicides and wood preservatives [109-110]. Their impact on the environment is of major concern because they are released in substantial quantities through solid and liquid effluents [111] and atmospheric discharges from municipal and industrial incinerators [112-117]. Chlorobenzenes can be carried long distances in the atmosphere and as a result of widespread uses over several decades they are found in water [118], soils [119-120], sediments [121], sewage sludges [122] and aquatic biota [123]. As chlorinated aromatic compounds are environmentally persistent and highly lipophilic this can lead to partitioning and/or accumulation in sediments and biota [124]. Monitoring their presence in the environment is important as their toxicity poses major

health risks. Chlorobenzenes have been found in the surface waters, bottom water and sediments of the Great Lakes basin (Canada) [125] and determined off the coast of Nagoya (Japan) close to an industrial area [126]. The highest concentration of chlorobenzenes were found in surface water and sediment. Higher substituted chlorobenzenes were found to have low concentrations in sea water and high concentrations in the sediment supporting the ideology that higher Kow's and lower solubilities are related to increased partitioning into hydrophobic medium. Di- and tri-chlorobenzenes were found to be present in all phases.

Scott et al. [67] and Mader et al. [127] have found that sorption of chlorobenzenes on sediment was controlled by both the electrostatic minerals present and partitioning into sediment organic matter associated with the minerals. Recent studies into the effect of temperature on Kow for chlorobenzenes indicated enhanced partitioning to lipid phases at low temperatures [128]. This suggests that animals in contact with, or living in cold sediments, are at greater risk of accumulating lethal amounts of hydrophobic chemicals. Bioaccumulation of sediment sorbed chlorobenzenes in the larval stages of the midge [129] showed rapid uptake from sediment into midge for all compounds, and their accumulation linearly related to the Kow's of chlorobenzenes. As midge larvae are major sources of foods for many fish species this is a potential threat to organisms higher in the food chain.

Chlorobenzene is considered one of the most difficult to incinerate of the US EPA's principle organic hazardous constituents [130]. This is primarily due to the strength of the C-Cl bond [131]. This may in part account for their resistance to abiotic and biotic degradation in the environment as substituents with chloro groups usually

complicate enzymatic attack [132]. However in recent studies bacteria have been described which are able to degrade tri- and tetra- chlorobenzenes [132-133] but no mention was made concerning the occurrence of these bacteria in the environment.

In 1991 as much as 0.25 mg/m^3 of 1,2,4-trichlorobenzene was measured in the air of Los Angeles [134] and concentrations ranging from 0.007 to 275 mg/l have been measured in the drinking water supplies of US cities. In the Maritime Provinces of Canada small amounts of chlorobenzenes have been found in sea birds [135] which commonly indicates a bioconcentration occurring through the food chain.

As the number of Cl atoms increases in the chlorobenzene series the vapour pressure and solubility decrease. There is also a trend of increased exothermic enthalpy of partitioning from water into organic carbon (Koc) and octanol (Kow), leading to greater persistence in environmental matrices [136-138] and bioaccumulation in living organisms. This accumulation may reach a level of toxicity detrimental to the well being of plants and animals [136, 139]. Increased partitioning of chlorobenzenes is also evident from the increasing retention times with increased Cl substitution or Kow in any GC or HPLC chromatogram separation on a hydrophobic stationary phase [140]. This is also true for hydrophobic polymeric sorbents such as Amberlite, XAD-2 and XAD-4 resins, where the degree of chlorine substitution increases the absorptive capacity of the aromatic polymeric sorbent increases [141].

Hydrophobic chemicals tend to accumulate in fat tissues for a long time without being metabolised [142]. The amount accumulated is related to the size of the fat cells and fat content, which therefore determines the lethal body burden. Accumulation of

pollutants in hydrophobic phases within organisms diminishes their ability to react to stimuli leading to narcosis and ultimately death due to influencing the fluidity of the membrane lipids, or competitively binding to the membrane proteins thus inhibiting their functions [143]. The bioconcentration of organic compounds has been found to increase between log Kow 2.5 and 6.0 [144]. However, compounds with a log Kow > 6 do not bioconcentrate as much as predicted from the extrapolation of linear relationships between the logarithm of the bioconcentration factor and Kow. This may be due to metabolisation to excretable hydrophilic compounds or as has been suggested elsewhere, reduced membrane permeation due to hindrance from molecular size [145].

The bioaccumulation of chlorobenzenes in both animals [146] and plants [147] has been determined by several research groups [140, 148]. An increase in the bioconcentration of chlorobenzenes in soy bean roots has been correlated with water-lipid partitioning and increased Kow [149]. Bioaccumulation factors of chlorobenzenes in species of fish and crabs [150], were found to be high due to slow metabolism rates in invertebrates [151] and vertebrate aquatic organisms [152]. The lethal body burden (LBB) of chlorobenzenes in guppies was found to be 2-2.5 mmol/kg organism [153] and for fathead minnows the lowest observable effect concentration for chlorobenzenes was between 0.47-5.1 mmol/kg organism [154]. Membrane burden of chlorobenzenes for lethality has been calculated to be 40-160 mmol/kg membrane [144]. Chlorobenzene accumulation in rainbow trout was 0.0299 mmoles/kg after 119 days, without any fish mortality [155] however this is probably due to the low concentration administered.

In juvenile crabs the bioconcentration factor, uptake and metabolism rate constants of chlorobenzenes showed correlation with Kow [156]. Concluding that

octanol/water is a reliable indicator of the partitioning tendency from water to organic media such as lipids proving that the major process for bioaccumulation of hydrophobic chemicals is partitioning between lipid phase and water [148]. As found with crabs the metabolism rates for chlorobenzenes also decrease with increase Kow for mammalian species [157]. In an experiment into the use of long term physiological bio-marking agents to research coyote behaviour 1,2,4,5-tetrachlorobenzene and pentachlorobenzene were administered to coyotes and were detected in the faeces and adipose tissue for 6 months after dosing [158], highlighting the potential for build up to levels of concern in mammalian species.

Other chlorinated compounds have similar action in the environment to chlorobenzenes. Pentachlorophenol is a metabolite of pentachlorobenzene in mammals and fish [158] and has also been found to persist in the environment at a high concentration for long periods of time [98]. Although elimination of pentachlorophenol in fish, rats and humans is similar to the detoxification of non-chlorinated phenolic compounds (via conjugation with glucuronic and sulfonic acids), its high protein binding in humans leads to slow excretion and long term exposure can cause liver damage [159]. Lipophilic organochlorine pesticides such as lindane (hexachlorocyclohexane) are released in huge quantities and slowly degraded in the environment. As a result they have been found in the air, sea, rivers, milk, human and animal food, and aquatic organisms. Organochlorine pesticides have also been found to bio-concentrate in the adipose tissue, milk and liver of humans [160]. Chlorophenols have been found to be mammalian metabolites of lindane.

Concern has therefore lead to the inclusion of chlorobenzenes into both the US EPA's [161, 66] and the European Communities [135] toxic pollutant lists as priority pollutants. Since chlorobenzenes are now routinely analysed by environmental laboratories, the requirement to generate reference materials to monitor the precision and accuracy of data generated within the laboratory and between laboratories has lead to the recent development of the first lake sediment reference material for determination of chlorobenzenes [162]. No sign of loss by degradation over 4 years of cold storage has been observed. Undoubtedly further reference materials for chlorobenzenes will be prepared with other matrices such as aquatic organisms.

1.7.3 Polyaromatic hydrocarbons (PAH's)

PAH's can enter the environment in several ways. As by-products of open fires or refuse burning, coal tar, coke tars or coke oven emissions, creosote, mineral oils, bitumens, industrial smoke and soot, cigarette and cigar tobacco smoke, tar or smoke condensates, and charcoal-broiled foods [163]. Vehicular exhaust emissions from gasoline or diesel motors are major PAH contributors, which are ubiquitous in urban air, and can therefore be inhaled. Other primary routes of potential human exposure to PAH's are inhalation of tobacco smoke, ingestion of contaminated water and foodstuffs. Since PAH's are toxic byproducts of many combustion processes, the ability to monitor these components in air, soil and water has significant consequences, as exposure to this wide variety of mixtures has been considered to be associated with the increased incidence of cancer in humans.

PAH's are non-polar and hydrophobic, thus their aqueous solubilities are low and decrease as the number of rings increases. PAH's are slightly less soluble in sea water than in fresh water due to the salting out effect. PAH bio-degradation is likely to occur at a faster rate in contaminated environments than in pristine environments because high PAH concentrations provide the opportunity for development of hydrocarbon degrading microbial populations. Naphthalene is a major component of pesticides, fungicides and dyes and is a commonly found pollutant in the environment. Naphthalene is one of the most biodegradable of the PAH's in oxygenated waters where it is attacked by bacteria and oxidised eventually to CO_2 and water. In the animal body some PAH's are converted to phenols [41] whereas others can accumulate. Correlations between soil organic matter content and the sorption of PAH's have found linear sorption isotherms over large concentration range, indicating partitioning [163].

Wood preserving creosote, a coal tar distillate, is composed of 90 % PAH's and the long term accumulation of creosote wastes at a wood-preserving facility has been studied [164]. In this case, in spite of the heavy contamination of stream sediments, none of the PAH compounds were detected in water samples. Walters and Luthy [165] found that partitioning from water increases onto activated carbon with decreased solubility and increased Kow. PAH's have been quantified in sediment cores by a lengthy Soxhlet extraction and clean up procedure and analysed by both HPLC and GC [166]. The levels of PAH's were related historically to motor vehicle activity and road runoff. Sorptive exchange with the aqueous phase was also found to be restricted. Higher hydrophobic PAH's concentrate in sediments and molluscs whilst accumulation of naphthalene is negligible, which relates to it's highest solubility and lowest Kow of all the PAH's.

An estimation of 50,000 gallons of coal derived oil containing a high concentration of PAH's and phenols will be released as an accidental spill into freshwater every 28 years [167]. Recent spillages include the infamous 'Exxon Valdez' oil spill on March 24th 1989 where 258,000 barrels of crude oil were released into the marine environment resulting in an impact on 780 km of the Prince William Sound shoreline [168]. Also, in 1993, 84,700 tonnes of middle crude oil (mainly naphthalenic: 74.6 %) was released into the coastal region off South Shetland resulting in a great impact on the environment [169]. Most water soluble compounds in coal liquids are readily degradable by natural bacterial communities. However, the insoluble residue of the oil can cause physical damage to birds and aquatic organisms and persist in the sediment for long time, resulting in chronic ecological damage through accumulation in tissues of plants and animals. Aquatic organisms inhabiting sediment containing coal liquid residues accumulate PAH's to levels exceeding sediment concentrations [167].

PAH's in the air have been found to be mainly associated with particles. PAH's partition appreciably into particulate matter and can be carried great distances from the source of pollution. Particulate matter is a complex mixture of soot, ashes, dirt, dust, pollens, molds and other carbon-based particles and acid aerosols. Pederson and Ingwersen [170] found that heavier PAH's were bound to particles below 1 μm, while lighter PAH's were substantially in the vapour phase. A significant proportion of diesel emission particulates have aerodynamic diameters smaller than 1 μm, and a higher burden of toxins than large ones. Larger particles may contain up to 4000 individual spherical particles clustered as agglomerates [171-173], which are often too heavy to be airborne for any length of time and pose a lower threat.

Of particular concern to human health are particulates in the inhalable size ranges referred to as PM_{10}'s (no larger than 10 μm in diameter). These particles readily enter the lungs with large particles tending to impact on the upper regions of the lung whilst smaller particles penetrate into the pulmonary region. The smaller particles can diffuse to the surface of the alveoli [174] where they become trapped and are thought to lead to serious health effects such as heart disease and cancer. PM_{10}'s are produced by diesel engines in particular i.e. taxis, lorries and busses. It is known that combustion-derived aerosol consists of solid carbonaceous soot particles (black carbon) that are associated with a complex mixture of organic compounds such as sorbed or condensed hydrocarbons and their derivatives. Particle bound PAH's are of particular concern because several have been demonstrated to cause mutations and certain types of cancer [175-177]. For example, benzo[a]pyrene accounts for a significant fraction of the mutagenic activity of urban aerosol extracts and has been shown to cause cultured cells to mutate and cancers to form in animal assays [178].

There is increasing concern that some anthropogenic chemicals present in water and air (free or attached to particles) may affect the endocrine systems (which regulate the action of hormones in organisms) of wildlife and humans leading to adverse health effects such as increased rates of specific cancers, immune system deficiencies and reproductive system abnormalities [179] such as sex changes in marine animals and the infamous falling sperm count in man. The "Department of Pharmacology and Toxicology, at the University of Western Ontario, Canada" have recently found evidence linking increased oestrogen activity to some PAH's (including anthracene) bound to PM_{10} air particulate material [180]. They suggested that carcinogenic PAH's possess electronic and steric configurations similar to steroid hormones. PAH's may therefore act in promoting the

development of hormone-dependent carcinogenesis and may interfere with steroid function by eliciting their carcinogenic effects by acting at the same sites as steroid hormones.

1.8 Summary and conclusions

As well as the above mentioned chemicals, there are numerous other pollutants entering, and having an impact on the environment from many sources. For example, pharmaceutical hormones from birth control pills which are excreted in the urine have recently been detected in sewage [181]. Heavy duty laundry powders, household cleaners and personal care products contain nonionic surfactants 'alkylphenol polyethoxylates' which can breakdown in the environment to form alkylphenols (such as nonylphenol and octylphenol). These compounds are endocrine-disrupting chemicals that can mimic oestrogen and have been linked with hormonally mediated toxic effects (reproductive problems) in fish down stream of sewage discharge points [182].

Butyltin compounds are thought to be another class of endocrine disrupting chemicals. Although elemental tin and its inorganic compounds have minor toxicity due to their low solubility in lipids organotin compounds such as tributyltin and triphenyltin have high bioconcentration factors in the livers of marine mammals [183]. They are used as anti-fouling agents in paints for boats and have been shown to cause physiological abnormalities such as sex change in gastropods and whelks [184-185].

Agriculturally applied chemicals have also attracted a great deal of attention due to their wide spread use to control weeds and pests. These classes of chemicals are of particular concern as they are applied purposefully to the environment in large quantities

and so can be found in both surface and ground water. Their degradation products are also cause for concern.

The unavoidable release and exposure of mixtures of potentially toxic chemicals in the urban environment is primarily the most worrying factor concerning our continued health. Conversely, the accidental release of pollutants is the most worrying to ecosystems due the high concentrations encountered close to the source, whilst some chemicals may be carried longer distances and impact on other ecosystems. Alteration of the composition of estuarine communities by pollution may affect ecological relationships amongst populations that finally could interrupt community stability.

Moderate amounts of phenols in the environment are degraded or are incorporated into humic substances whilst chlorobenzenes and PAH's are more likely to be persistent and bio-accumulate due to their high hydrophobicity. The wide range of different chemicals released (whether toxic or not) and their environmental degradation products by soil/sediment degradation, animal metabolism or photo degradation pose a great threat to health. With the threat of global warming, increasing temperatures may lead to an increase in reaction rates of chemicals in environmental systems as well as UV-light catalysing photo degradation and causing the formation of free radicals. These factors provide the potential for unimaginable reactions to take place which may produce highly toxic chemicals.

Being exposed to a 'cocktail' of low levels of chemicals may well have a more adverse affect than the same level of a single chemical. In vitro assays which involve single cell type cultures are simple, and capable of screening large numbers of samples. In

addition, these assays are valuable because they are able to indicate a potential mechanism of action as well as account for synergistic, antagonistic, and additive interactions that may occur within a complex mixture. However, 'in vitro' assays may not be able to take into account the wider implications of other mechanisms of action that may lead to adverse effects. Important factors such as bioconcentration and metabolism v's bioaccumulation can not be modeled 'in vitro'.

It may be that a combination of chemicals in the bodily environment can act synergistically to promote disease or, for example, effect reproduction. Due to limitations on time, complexity and difficulty in data interpretation, little work is actually done on the adverse physiological effects that 'cocktails' from the same and/or different groups of chemicals have on plants and animals. At present the best solution is to control the source.

Although many chemicals can be degraded or incorporated into soil or sediments, the effect that this has on soil properties such as change in pH may adversely effect the biological functioning of plants, algae and aquatic organisms. For these reasons it is important to regulate the amount of these chemicals we consume and therefore continual environmental monitoring is necessary coupled with highly sensitive methods of analysis, detection and characterisation.

1.9 References

1. P.M. Fedorak and S.E. Hrudey, In '*Anaerobic degradation of phenolic compounds with applications to treatment of industrial waste waters*'; Biotreatment Systems CRC Press: Boca Raton FL, Chap. 4, **vol.1** (1988) 169.
2. S.M. Lambert, P.E. Porter and R.H. Schieferstein, *Weeds,* **13** (1965) 185.
3. M.T. Suidan, C.E. Strubler, S-W. Kao and J.T. Pfeffer, *J. Wastewater Pollut. Control Fed.,* **55** (1983) 1263.
4. S. Dagley, *Dev. Ind. Microbiol.,* **25** (1984) 53.
5. Anonymous, *Chem. Eng. News,* **66** (1988) 34.
6. Z.A. Grosser, J.F. Ryan and M.W. Dong, *J. Chromatogr.,* **642** (1993) 75.
7. V.D. Radisav, M.T. Suldan and R.D. Brenner, *Environ. Sci. Technol.,* **27** (1993) 2079.
8. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.,* **66** (1994) 160.
9. E.R. Graber and M.D. Borisover, *Environ. Sci. Technol.,* **32** (1998) 258.
10. W.Y. Shiu and D. Mackay, *J. Chem. Eng. Data,* **42** (1997) 27.
11. G.A. Robbins, S. Wang, and J.D. Stuart, *Anal. Chem.*, **65** (1993) 3113.
12. L.S. Lee, P. Suresh, C. Rao, P. Nkadi-Kizza, and J.J. Delfino, *Environ. Sci. Technol.,* **24** (1990) 654.
13. E.A. Maier, A. Booenke and P. Meriguet, *Trends in Anal. Chem.*, **16** (1997) 496.
14. R. Walker, *Valid Analytical Measurement (VAM) Bulletin*, **18** (1998) 5.
15. International Vocabulary of Basic and General Terms in Metrology, (1993 Edn).
16. K. Meyer, P. Klobes and B. Rohl-Kuhn, *Cryst. Res. Technol.,* **32** (1997) 175.
17. R. Alvarez, *The Science of the Total Environment.,* **104** (1991) 1.
18. M.P. Llompart, R.A. Lorenzo and R. Cela, *J. Chromatogr. A,* **723** (1996) 123.
19. V.L- Avila, R. Young and W.F. Berkert, *Anal. Chem.*, **66** (1994) 1097.

20. A. Egizabal, O. Zuloaga, N. Etxebarria, L.A. Fernandez and J.M. Madariaga, *Analyst*, **123** (1998) 1679.

21. U. Faure and P.J. Wagstaffe, *Fresenius J. Anal. Chem.*, **345** (1993) 124.

22. F. C. Spurlock and J.W. Biggar, *Environ. Sci. Technol.*, **28** (1994) 989.

23. W.P. Ball and P.V. Roberts, *Environ. Sci. Technol.*, **25** (1991) 1223.

24. S.W. Karickhoff, *J. Hydraul. Eng.*, **110** (1984) 770.

25. C.T. Chiou and D.E. Kile, *Environ. Sci. Technol.*, **28** (1994) 1139.

26. U. Mingelgrin and Z. Gerstl, *J. Environ. Qual.*, **12** (1983) 1.

27. J.P.M. Vink and Sjoerd E.A.T.M. van der Zee, *Pestic. Sci.*, **46** (1996) 113.

28. T.S.C. Wang, S.W. Li, and Y.L. Ferng, *Soil Sci.*, **126** (1978) 15.

29. J.M. Bollag, '*Cross coupling of humus constituents and Xenoboitic substances*'; Aquatic and terrestrial humic materials; R.F. Christman and E.T. Gjessing, Eds.; **chap. 6** (1983) 127.

30. J.P. Martin and K. Haider, *Soil Sci.*, **107** (1969) 260.

31. S. Chen, W.P. Inskeep, S.A. Williams and P.R. Callis, *Soil Sci. Soc. Am. J.*, **56** (1992) 67.

32. R.D. Sjoblad and J.M. Bollag, *Appl. Environ. Microbiol.*, **33** (1977) 906.

33. J.P. Martin, K. Haider and D. Wolf, *Soil Sci. Am. Proc.*, **36** (1972) 311.

34. G. Knoll and J. Winter, *Appl. Microbiol. Biotechnol.*, **25** (1987) 384.

35. W. J. Smolenski and J.M. Suflita, *Appl. Environ. Microbiol.*, **53** (1987) 710.

36. F. Bak and F. Widdel, *Arch. Microbiol.*, **146** (1986) 177.

37. O.A. O'Connor and L.Y. Young, *Environ. Toxicol. Chem.*, **8** (1989) 853.

38. H.D. Scott, D.C. Wolf, and T.L. Lavy, *J. Environ. Qual.*, **11** (1982) 107.

39. J.B. Healy, Jr. and L.Y. Young, *Appl. Environ. Microbiol.*, **35** (1978) 216.

40. M.M. Haggblom, M.D. Rivera and L.Y. Young, *Appl. Environ. Microbiol.*, **59** (1993) 1162.

41. M.H. Rogoff, *Adv. Appl. Microbiol.*, **3** (1961) 193.

42. S. Burchill, M.H.B. Hayes and D.J. Greenland, In '*The Chemistry of Soil Processes*'; D.J. Greenland and M.H.B. Hayes, Eds.; John Wiley & Sons Ltd., **Chap. 6** (1981) 221.

43. D.R. Garbarini and L.W. Lion, *Environ. Sci. Technol.*, **20** (1986) 1263.

44. P. Grathwohl, *Environ. Sci. Technol.*, **24** (1990) 1687.

45. S.J. Severtson and S. Banerjee, *Environ. Sci. Technol.*, **30** (1996) 1961.

46. W. Karcher, G. Kuhnt, M. Herrmann and H. Muntau, Proceedings of the 5th International Workshop Environmental Behaviour of Pesticides and Regulatory Aspects, A. Copin, G. Houins, L. Pussemier, J.F. Salembier, Eds.; Brussels (1994), April 26-29, p112.

47. L.S. Lee, P. Suresh, C. Rao, and M.L. Brusseau, *Environ. Sci. Technol.*, **25** (1991) 722.

48. J.J.T.I. Boesten, *Pestic. Sci.*, **30** (1990) 31.

49. L.Y. Young and A.C. Frazer, *Geomicrobiology Journal*, **5** (1987) 261.

50. F.J. Stevenson, In '*Humus chemistry* ', Wiley: New York (1982).

51. F.J. Stevenson, In '*Methods of soil analysis: Part 2*'; American Society of Agronomy: Madison, WI, (1965) 1409.

52. P.I. Dem'Yanov, 'SAR and QSAR in Environmental Research', Presented at the 6th International Workshop on Quantitative Structure-Activity Relationships (QSAR) in Environmental Sciences, September 13-17, Belgirate, Italy, **4** (1995) 221.

53. S.A. Boyd, M.M. Mortland and C.T. Chiou, *Soil Sci. Soc. Am. J.*, **52** (1988) 652.

54. H.J. Wirth and M.T.W. Hearn, *J. Chromatogr. A*, **711** (1995) 223.

55. T.M. Young and W.J. Weber, Jr., *Environ. Sci. Technol.*, **29** (1995) 92.

56. H. R. Schulten, *Fresenius J. Anal. Chem.*, **351** (1995) 62.

57. Z. Wang, B.C. Pant and C.H. Lanford, *Anal. Chim. Acta*, **232** (1990) 43.

58. H.J. Wirth and M.T.W. Hearn, *J. Chromatogr. A*, **711** (1995) 223.

59. G. Chesters and D.A. Graetz, *In 'Chromatographic analysis of the Environment'*; R.L.Grob, Ed.; Marcel Dekker, Inc., 270 Madison Avenue, New York, 10016 (1975).

60. L.B. Barber, E.M. Thurman and D.D. Runnells, *J. Contam. Hydrol.*, **9** (1992) 35.

61. K.Othmers, Encyclopedia of chemical technology, **8** (1993) 108.

62. J. Poulstka, K. Holadova and J. Hajslova, *Intern. J. Environ. Anal. Chem.*, **60** (1995) 139.

63. C. Yu and D. Crump, *Building and Environment*, **33** (1998) 357.

64. K-D. Wenzel, A. Hubert, M. Manz, L. Weissflog, W. Engewald and G. Schuurmann, *Anal. Chem.*, **70** (1998) 4827.

65. B. Xing, W.B. McGill and M.J. Dudas, *Environ. Sci. Technol.*, **28** (1994) 466.

66. L.H. Keith and W.A. Telliard, *Environ. Sci. Technol.*, **13** (1979) 416.

67. H.D. Scott, D.C. Wolf and T.L. Lavy, *J. Environ. Qual.*, **11** (1982) 107.

68. J.M. Bollag, R.J. Sjoblad, E.L. Czaplicki and R.E. Hoeppel, *Soil Biol. Biochem.*, **8** (1976) 7.

69. J.M. Davidson and J.R. McDougal, *J. Environ. Qual.*, **2** (1973) 428.

70. J.M.N. Sieburth and A. Jensen, *J. Exp. Mar. Biol. Ecol.*, **3** (1969) 275.

71. A.T. Stone, *Environ. Sci. Technol.*, **21** (1987) 979.

72. S.A. Boyd, *Soil. Sci.*, **134** (1982) 337.

73. G.P. Curtis, P.V. Roberts and M. Reinhard, *Water Resour. Res.*, **22** (1986) 2059.

74. L.T. Ou and H.C. Sikka, *J. Agric. Food Chem,* **25** (1977) 1336.

75. C.C.R. Allen, D.R. Boyd, M.J. Larkin, K.A. Reid, N.D. Sharma and K. Wilson, *Appl. Environ. Microbiol.,* **63** (1997) 151.

76. S. Chen, W.P. Inskeep, S.A. Williams and P.R. Callis, *Soil Sci. Soc. Am. J.*, **56** (1992) 67.

77. K.A. Massey, D.L. Van Engelen and I.M. Warner, *Talanta.,* **42** (1995) 1457.

78. R.D. Sjoblad, R.D. Minard and J.M. Bollag, *Pestic. Biochem. Physiol.,* **6** (1976) 457.

79. D.B. Shealy, J.R. Barr and D.L. Ashley, *Environmental Health Perspectives*, **105** (1997) 510.

80. O.A. O'connor and L.Y. Young, *Environ. Sci. Technol.,* **30** (1996) 1419.

81. A. Tschech and G. Fuchs, *Arch. Microbiol.,* **148** (1987) 213.

82. J.P. Gould and W.J. Weber, Jr., *J. Water Pollut. Control Fed.,* **48** (1976) 47.

83. P.C. Chrostowski, A.M. Dietrich and I.H. Suffet, *Water Res.,* **17** (1983) 1627.

84. B.M. van Vliet and W.J. Weber, Jr., *J. Water Pollut. Control Fed.,* **53** (1981) 1585.

85. C.R. Fox, *hydrocarbon processing,* (1975) 109.

86. E.H. Crook, R.P. McDonnell and J.T. McNulty, *Ind. Eng. Chem., Prod. Res. Dev.,* **14** (1975) 113.

87. F-D. Kopinke, J. Porschmann and U. Strottmeister, *Environ. Sci. Technol.* ,**29** (1995) 941.

88. C.W. Carter and I.H. Suffet, *Environ. Sci. Technol.,* **16** (1982) 735.

89. Y-P. Chin and W.J. Weber, Jr., *Environ. Sci. Technol.,* **23** (1989) 978.

90. P.J. Isaacson and C.R. Frink, *Environ. Sci. Technol.,* **18** (1984) 43.

91. S. Chen, W.P. Inskeep, S.A. Williams and P.R. Callis, *Environ. Sci. Technol.*, **28** (1994) 1582.

92. M.J. Morra, M.O. Corapcioglu, R.M.A. von Wandruszka, D.B. Marshall and K. Topper, *Soil Sci. Soc. Am. J.*, **54** (1990) 1283.

93. W.D. Burgos, J.T. Novak, and D.F. Berry, *Environ. Sci. Technol.*, **30** (1996) 1205.

94. J.P. Martin, K. Haider and D. Wolf, *Soil Sci. Soc. Am. Proc.*, **36** (1972) 311.

95. A.M. Anselmo, J.M.S. Cabral and J.M. Novais, *Appl. Microbiol. Biotechnol.*, **31** (1989) 200.

96. H. Babich and D.L. Davis, *Regul. Toxic. Pharmacol.*, **1** (1981) 90.

97. B.J. Dean, *Mutation Research,* **47** (1978) 75.

98. Ulf G. Ahborg, Jon E. Zinc and T.M. Thunberg, *CRC Crit. Rev. Toxicol.*, **7** (1980) 1.

99. S. Kobayashi, M. Moriwaki, I. Hachiya, *Tetrahedron Letters.*, **37** (1996) 4183

100. K. Ding and Q. Xu, *Chem. Commun.*, **7** (1997) 693.

101. J.M. Bollag, R.J. Sjoblad, E.L. Czaplicki and R.E. Hoeppel, *Soil Biol. Biochem.*, **8** (1976) 7.

102. D. Bianchi, A. Bernardi, A. Bosetti and R. Bortolo, *Appl. Microbiol. Biotechnol.*, **48** (1997) 363.

103. T.S.C. Wang, S. W. Li, and Y.L. Ferng, *Soil Sci.*, **126** (1978) 15.

104. R.D. Sjoblad, R.D. Minard and J.M. Bollag, *Pestic. Biochem. Physiol.*, **6** (1976) 457.

105. R.D. Sjoblad and J.M. Bollag, *Appl. Environ. Microbiol.*, **33** (1977) 906.

106. F.J. Santos, M.N. Sarrion and M.T.Galceran, *J. Chromatogr. A*, **771** (1997) 181.

107. J.E.M. Beurskens, C.G.C. Dekker, H.van den Huevel, M.Stuart, J.deWold and J.Dolfing , *Environ. Sci. Technol.*, **28** (1994) 701.

108. Y.F. Li and E.C. Vordner, *Sci. Total Environ.*, **160** (1995) 201.

109. HSDB. 1994. Hazardous Substances Data Bank. MEDLARS Online Information Retrieval System, National Library of Medicine.

110. N.I. Sax and R.J. Lewis, Sr., Eds. In '*Hawley's Condensed Chemical Dictionary*', 11th Edn. New York: Van Nostrand Reinhold Company, (1987).

111. B.G. Oliver and K.D. Nicol, *Environ. Sci. Technol.*, **16** (1982) 532.

112. C.R. Pearson, In '*The handbook of Environmental chemistry, Anthropogenic compounds*', Ed.; B. Hutzinger, Part B. Springer, Berlin Heidelberg, New York, **1** (1982).

113. C.G. Choudhry and O. Hutzinger , *Environ. Sci. Technol.*, **18** (1984) 235.

114. G.G. Choudhy, and O. Hutzinger, *Toxicol. Environ. Chem.*, **5** (1982) 1.

115. K. Olie, P.L. Vermeulen and O. Hutzinger, *Chemosphere.*, **6** (1977) 455.

116. G.A. Eiceman, R.E. Clement and F.W. Karasek, *Anal. Chem.*, **51** (1979) 2343.

117. E.B. Lunden and G. Lovblad, *Atmos. Environ.*, **25A**.(1991) 2251.

118. S.Franke, S.Hildebrant and W.Franke, *Fresenius J. Anal. Chem.*, **353** (1995) 39.

119. A.J. Sweetman and C.D. Watts, *Environ.Technol.*, **16** (1995) 73.

120. A.J. Beck, D.L. Johnson and K.C. Jones, *Sci. Total Environ.*, **185** (1996) 125.

121. M.T. Prytula and S.G. Paulostathis, *Wat. Sci. Technol.*, **33** (1996) 247.

122. M.J. Wang, S.P. Grath and K.C. Jones, *Environ. Sci. Technol.*, **29** (1995) 356.

123. A.P. Vanwezel and A. Opperhuizen, *Chemosphere.*, **31** (1995) 3605.

124. E.E. Kenaga and C.A.I. Goring, 3rd Aquatic Toxicology Symposium Proceedings of the American Society for Testing and Materials, **707** (1978) 78.

125. B.G. Oliver, *Water Pollut. Res. J. Can.*, **19** (1984) 47.

126. S. Masunaga, Y. Urushigawa and Y. Yonezawa, *Wat. Res.*, **25** (1991) 289.

127. B.T. Mader, K.U. -Goss and S.J. Eisenreich, *Environ. Sci. Technol.*, **31** (1997) 1079.

128. N.P. Bahadur, W.Y. Shiu, D.G.B. Boocock and D. Mackay, *J. Chem. Eng. Data.*, **42** (1997) 685.

129. J.P. Knezovich and F.L. Harrison, *Ecotoxicol. Environ. Safety,* **15** (1988) 226.

130. R. Morlando and S.E. Manahan, *Environ. Sci. Technol.*, **31** (1997) 409.

131. J.L. Graham, D.L. Hall and B. Dellinger, *Environ. Sci. Technol.*, **20** (1986) 703.

132. S. Beil, B. Happe, K.N. Timmis and D.H. Pieper, *Eur. J. Biochem.*, **247** (1997) 190.

133. P. Sander, R.M. Wittich, P. Fortnagel, H. Willas and W. Francke, *Appl. Environ. Microbiol.*, **57** (1991) 1430.

134. IPCS. 1991. International Programme of Chemical Safety. Environmental Health Criteria 128. Chlorobenzenes other than hexachlorobenzene. World Health Organization, Geneva, Switzerland, p252.

135. R.A.F. Matheson, E.A. Hamilton, A. Trites and D. Whitehead, Surveillance Report EPS-5-AR-80-1, Environment Canada, Environmental Protection Service, Halifax, Nova Scotia, Canada, March. (1980).

136. M.Th.M. Tulp and O. Hutziger, *Chemosphere,* **7** (1978) 849.

137. TR192.1994. (1992) Toxics Release Inventory. Office of Pollution Prevention and Toxics, US EPA, Washington DC, p84.

138. D. Makay, W.Y. Shiu and K.C. Ma, (1992) In '*Illustrated handbook of physical - chemical properties and environmental fate for organic chemicals*'. Vol.**1**., Lewis Publishers, Boca Ranton.

139. W.N. Beyer, *Bull. Environ. Contam. Toxicol.*, **57** (1996) 729.

140. A. Opperhuizen, *Toxicol. Environ. Chem.*, **15** (1987) 249.

141. R. Kunin, *Pure and Appl. Chem.*, **46** (1976) 205.

142. K. Umegaki, H. Uramoto, J. Suzuki and T. Esashi, *Jpn. J. Toxicol. Environ. Health,* **42** (1996) 12.

143. A.P. Van Wezel, G. Cornelissen, J. Kees, V. Miltenburg and A. Opperhuizen, *Environ. Toxicol. Chem.*, **15** (1996) 203.

144. M. Chessells, D.W. Hawker and D.W. Connell, *Ecotoxicol. Environ. Safety,* **23** (1992) 260.

145. W. de Wolf, J.H.M. de Bruljn, W. Selnen and J.L.M. Hermens, *Environ. Sci. Technol.*, **26** (1992) 1197.

146. B.G. Oliver and K.D. Bothen, *Intern. J. Environ. Anal. Chem.*, **12** (1982) 131

147. I. Scheunert, E. Topp, A.Attar and F. Korte, *Ecotoxicol. Environ. Safety,* **27** (1994) 90.

148. A.P. van Wezel and A. Opperhuizen, *CRC Crit. Rev. Toxicol.*, **25** (1995) 255.

149. H. Kraaij and D.W. Connell, *Chemosphere,* **34** (1997) 2607.

150. L.P. Burkhard, B.R. Sheedy, D.J. McCauley and G.M. DeGraeves, *Environ. Toxicol. Chem.*, **16** (1997) 1677.

151. R.F. Lee, *Mar. Environ. Res.*, **28** (1989) 93.

152. J.R. Sunborn, W.F. Childers and L.G. Hansen, *J. Agric. Food Chem.*, **25** (1977) 551.

153. G.J.V. Hoogen and A. Opperhuizen, *Environ. Toxicol. Chem.*, **7** (1988) 213.

154. A.R. Carlson and P.A. Kosian, *Arch. Environ. Contam. Toxicol.*, **16** (1987) 129.

155. A.J. Niimi and L.L. -Jinde, *Arch. Environ. Contam. Toxicol.*, **13** (1984) 303.

156. M.R. Mortimer and D.W. Connell, *Aust. J. Mar. Freshwater Res.*, **44** (1993) 565.

157. H.B. Mathews, W.B. Jakoby, J.R. Bend and J. Caldwell eds. In *'Metabolic Basis of Detoxification. Metabolisation of functional groups'*; Academic. New York NY. USA, (1982) p51.

158. J.J. Johnston, C.A. Furcolow, O.U.R. Terry and B.A. Kimball, *Pestic. Sci.,* **50** (1997) 249.

159. M. Brandt., E. Schmidt, D.R. Zchivago and F.W. Schmidt, Chronische Lebererkrannkung durch langjahrige Intoxikation in Haushalt mit Pentachlorophenol., *Verh. Dtsch. Ges. Inn. Med.*, **83** (1977) 1609.

160. T.H.E. Gaffer, I. Scheunert and F. Korte, *Chemosphere,* **16** (1987) 239.

161. US Environmental Protection Agency. Health Assesment Document for Chlorobenzenes, EPA-600/8-84-015F, Washington DC (1985).

162. H.B. Lee, R.L.H. -You and A.S.Y. Chau, *Analyst.,* **111** (1986) 81.

163. S.W. Karickhoff, D. S. Brown and T. A. Scott, *Water Res.*, **13** (1979) 241.

164. J.F. Elder and P.V. Dockers, *Environ. Pollut.,* **49** (1988) 117.

165. R.W. Walters and R.G. Luthy, *Environ. Sci. Technol.*, **18** (1984) 395.

166. J.W. Readman and B. Badger, *Sci. Total Environ.,* **66** (1987) 73.

167. J.M. Giddings, S.E. Herbes and C.W. Gehrs, *Environ. Sci. Technol.,* **19** (1985) 14.

168. A. Maki, *Environ. Sci. Technol.*, **25** (1991) 24.

169. L. Webster, L. Angus, G. Topping, E.J. Dalgarno and C.F. Moffat, *Analyst*, **122** (1997) 1491.

170. P.S. Pederson and J. Ingwersen, *Environ. Sci. Technol.,* **14** (1980) 71.

171. U.R.A. Dirty and S.L. Uttpipe, Particulate matter Associated with Vehicles on the Road. II. *Aerosol Sci. Technol.,* **2** (1983) 1.

172. D.F. Dolan and D.B. Kittelson, *Study of Roadway Diesel Aerosols*; Society of Automotive Engineers, Technical Paper 790492, 1979.

173. W.H. Lipkea, J.H. Johnson and F. Kipper, *'The Physical and Chemical Character of Diesel Particulate Emmisions - Measurement Techniques and Fundamental Considerations'*; Society of Automotive Engineers, Technical Paper 791702, 1979.

174. US Environmental Protection Agency. *'Air quality criterion for particulate matter and sulfur oxides';* Technical Report EPA-600/8-82-029; US EPA: Washington, DC, 1982.

175. K.M. Nauss, Critical issues in assessing the carcinogenicity of diesel exhaust: a synthesis of current knowledge. In *'Diesel Exhaust: a critical analysis of emmissions, exposure, and health effects'*; Health Effects Institute: Cambridge, MA, 1995.

176. J.C. Means, S.G. Wood, J.J. Hassett and W.L. Banwart, *Environ. Sci. Technol.*, **14** (1980) 524.

177. C. Heidelberger, (1976) Studies on the Mechanism of Carcinogenisis by Polycyclic Aromatic Hydrocarbons and their Derivatives., In *'Carcinogenesis, A Comprehensive Survey'*; R.I. Fredenthal and P.W. Jones, Eds.; **Vol. 1**, Raven Press, New York, p1.

178. M.R. Osborn and N.T. Crosby, In *'Benzopyrenes'*; Cambrige University Press: Cambridge, 1987.

179. J.A. McLachlan and K.S. Korach, *Environ. Health Perspect.*, (Suppl.7) **104** (1995) 3.

180. J.H. Clemons, L.M. Allan, C.H. Marvin, Z. Wu, B.E. McCarry, D.W. Bryant and T.R. Zacharewski, *Environ. Sci. Technol.*, **32** (1998) 1853.

181. U.K. Environmental Agency. '*The Identification and Assesment of Estrogenic Substances in Sewage Treatment Works Effluents*'; R&D Technical Summary; U.K. Environmental Agency: (1996) 38.

182. L.C. Folmar, N.D. Denslow, V. Rao, M. Chow, D.A. Crain, J. Enblom, J. Marcino and L.J. Guilette, *Environ. Health Perspect.*, **104** (1996) 1096.

183. S. Tanabe, M. Prudente, T. Mizuno, J. Hasegawa, H. Iwata and N. Miyazaki, *Environ. Sci. Technol.*, **32** (1998) 193.

184. P.E. Gibbs, G.W. Bryan, P.L. Pascoe and G.R. Burt, *J. Mar. Biol. Assoc., U.K.*, **70** (1990) 639.

185. G.W. Bryan, P.E. Gibbs, L.G. Hummerstone and G.R. Burt, *J. Mar. Biol. Assoc., U.K.*, **66** (1986) 611.

Chapter 2.0

Determination of pollutants in the environment

2.1 Introduction

There's an increased demand on laboratories to produce rapid and inexpensive analysis of organic pollutants in the environment [1-2]. Sample preparation is the most labour intensive, time consuming and difficult task in the analysis of organic compounds due to the need to pre-concentrate low levels of specific target compounds with high recovery to aid sensitivity, precision and accuracy. Difficulty also arises as a result of removal of non-target co-extractives termed 'matrix interferences' [3-4].

Phenols and PAH's have been analysed in coal wastewater and sediments [5]. The severe difficulty when analyzing organic pollutants in environmental matrices such as these, lies in the complex mixtures of different compounds and co-extracted matrix interferences present such as humic acids. The EPA recognise that gas chromatography (GC) coupled with mass spectrometry (MS) is the only available technique that can identify a wide range of compounds in many different matrices and in the presence of interfering compounds. Their toxic pollutant list contains chlorobenzenes, phenols and PAH's [6].

Chromatography (formed from the Greek 'chroma' meaning colour and 'graphien' meaning to write) has become an integral technique of separation and analysis in the laboratory since its inception by Tswett around 1900 [7-8]. The traditional analysis technique for volatile organics used by the EPA is GC [9]. Electron-capture detection (ECD) is used extensively for pesticides, chlorinated compounds and phthalates. Nitrogen-phosphorus detectors are used for the analysis of nitrosamines, amines, acrylonitrile, and nitrogen- and phosphorus-containing pesticides. MS with confirmatory

capability is the preferred detector for survey methods and for screening samples and functions as a universal detector. Sensitivity and selectivity can be increased significantly by using MS in the single-ion monitoring (SIM) mode.

The EPA recognises the applicability of high performance liquid chromatography (HPLC) for non-volatile, thermally unstable, and polar chemicals [9]. With the trend towards biodegradable pesticides, HPLC is becoming the preferred analytical method for most insecticides and their metabolites, herbicides, and plant growth regulators. Key target pesticides include carbamates, diquat and paraquat, triazines and phenylureas. In addition HPLC is also amenable to acids, bases, surface active agents, dyes, and aromatic amines and can accommodate large volume injections. Traditional sample preparation procedures pose severe limitations on the overall performance of the method. With continual technological improvements being made to provide highly efficient chromatographic separations and detection systems there is a requirement for concomitant efficiencies in sample handling procedures.

The synthesis of high surface area polymers, commonly termed 'macroreticular resins' has lead to the development of a new class of sorbents with wide range of sorption behaviours in which organic compounds may be sorbed via 'van der Waal' interactions from their aqueous or non-aqueous environments [10-11]. For example, columns containing Amberlite XAD-2 and XAD-4 sorbents have been used to successfully remove barbiturates and glutethimide from the blood streams of comatose patients by hemoperfusion and were found to be clinically superior to hemodialysis in the treatment of drug intoxication [12-13]. Sorbents are generally solids that possess high specific surfaces, (usually well above 5 m^2/g), however, liquid like polymeric sorbents also have

specific applications as will be discussed later. Compared with ion exchange resins these sorbents are much easier to regenerate due to weaker bonding forces than the electrostatic attractions present in ion exchange resins.

Particular interest concerns the large scale use of these sorbents for the treatment of wastes from various industrial processes such as to removing pollutants from Kraft pulp mill effluents [14-17]. On a smaller scale polymer sorbents have found uses such as selective coatings in gas chromatography (GC), packings in high pressure liquid chromatography (HPLC) and solid phase extraction (SPE) [18] and now fibre coatings in solid phase microextraction (SPME).

Soil contains a mixture of different sorbent surfaces and 'liquid-like' polymer sorbents with varying properties. As a result soil is one of the most recalcitrant matrices known and its complexity has gained the attention of microbiologists through to hydraulic engineers. The emancipation of target analytes from the soil matrix for identification and quantitation is riddled with problems. These include diffusive rate limitations due to extensive soil pore structure [19-20], as well as soil ageing which may lead to organic compounds becoming increasingly chemically bound or physically trapped [21-22].

As concern grows towards the release of pollutants into the environment it is of paramount importance that we develop simple, environmentally friendly, efficient and inexpensive analytical methods in order to monitor our own and industries chemical releases [23-28]. To achieve this we must be able to extract and analyse pollutants in different matrices. This section describes the conventional methods of extraction of

organic compounds from their matrices alongside more recent developments in extraction methodologies.

2.2 Traditional sample preparation techniques

2.2.1 Liquid-solid extraction (LSE). Shake flask and Soxhlet

Soxhlet extraction, developed by Franz Ritter von Soxhlet, is the standard technique for the extraction of semi-volatile and non-volatile compounds from solids. In Soxhlet extraction the solid sample is mixed with a drying agent, such as, anhydrous sodium sulphate (which also acts to increase the solvent contact surface area of the sample) and placed in a cellulose thimble which acts as an inert solvent filter. The thimble is placed in a reflux apparatus above a round bottomed flask containing an organic solvent. The round bottomed flask is heated causing the solvent to be refluxed over the sample thus removing compounds from the solid matrix. As a result of solvent refluxing, the extraction process is at a lower temperature than the boiling point of the solvent. Any water released from the solid into the solvent is absorbed by the drying agent to prevent emulsions from forming. The extract is usually pre-concentrated by evaporation and may require further clean-up prior to analysis.

In shake flask extraction (LSE without heat), a solid is placed in a bottle with an extraction solvent, the bottle is sealed and shaken for a certain length of time, after which, the extract is filtered and like Soxhlet may require preconcentration and further clean-up procedures.

The EPA has 6 solid waste extraction methods including methylene chloride extraction, continuous liquid-liquid extraction, methylene chloride-acetone Soxhlet extraction, automated Soxhlet extraction, methylene chloride-acetone ultrasonic extraction and waste dilution [9]. The British Gas Method for the analysis of PAH's in soils by GC-MS uses Soxhlet extraction of 10-15 g soil with 100 ml dichloromethane (DCM) for 6 hrs followed by internal standard addition and SIM GC-MS [29].

The time consumption involved in the sample preparation steps centered around Soxhlet and shake flask methods is well demonstrated in the literature [30-31]. As these techniques are not necessarily compound specific, volatile through to non volatile compounds as well as elements such as sulfur can also be extracted. The choice of solvent may result in higher compound extraction specificity but also dictates the extraction temperature of the reflux which may lead to incomplete recoveries. As a knock on effect, Soxhlet extractions generally take long periods of time [32-33] resulting in limited sample throughput. These methods are also becoming increasingly unpopular due to the large quantities of expensive high purity solvents they require [34]. Extract and preconcentration methods such as Soxhlet can lower limits of detection (LOD's) but also concentrate impurities. Therefore the extract often requires extensive sample cleanup which may yield incomplete recovery and/or degradation of the analyte species [35-39].

2.2.2 Liquid-liquid extraction (LLE)

Rivers, lakes or the sea may contain large amounts of organic compounds due to the polluting activities of industries. However, due to massive dilution, water samples taken from these aqueous bodies will have very low levels of these pollutants. In order to

detect these levels quantitatively, large volumes of water are required to be pre-concentrated prior to analysis.

Liquid samples containing non-volatiles and semivolatile compounds are routinely extracted by either LLE or more recently solid phase extraction (see below) [40]. Like shake flask with solids, LLE simply involves shaking a water sample with an organic solvent to partition analytes between the two phases. The solvent is removed and usually pre-concentrated before analysis. This method of extraction also extracts a wide range of compounds present in the water sample which can interfere with quantitation of the target analytes. The requirement of a clean up stage post extraction has been highlighted by the poor chromatography observed by McKone et al. [41] who extracted triazine herbicides from pond and canal water with DCM. Lee and Stokker [42] removed matrix interferences from a methylene chloride extract of lake water containing 11 triazine herbicides by clean up with florisil and observed enhanced chromatography.

Recently liquid-liquid microextraction has been used which combines extraction and concentration steps thus eliminating the concentration step in conventional LLE. This method has been used to extract chlorobenzenes from 500-1000 ml water samples with 1-2 ml organic solvent [43-44]. In both cases salt was added prior to extraction, probably to reduce emulsions and enhance salting out into the small amount of solvent. Similar recoveries were reported by both groups and ranged from c.a. 75 % for dichlorobenzenes to c.a. 90 % for tetrachlorobenzenes.

In the past LLE has been shown to possess certain disadvantages. It can produce emulsions [44] and low extraction efficiencies for various compounds as well as using

large volumes of high purity solvents which eventually must be disposed of. Like LSE, LLE is costly and can be affected by solvent contamination problems and loss of analyte at low concentrations due to sample handling. It can also be labour intensive and time consuming (4-18 hr extraction, 20-45 min handling time per sample [45]) and is not easily automatable. During evaporation of large amount of solvent, loss of analytes such as chlorobenzenes may contribute to low recoveries [46]. Clean-up procedures require repeated manipulation of the sample and are therefore tedious, time consuming, and prone to procedural loss.

2.2.3 Purge and trap headspace analysis (P&T)

The EPA purge and trap method coupled to GC (based on the procedure of Bellar and Lichtenberg [47]) is the approved technique in the USA for on-line extraction and concentration for the determination of volatile organic compounds (VOC's) in aqueous samples and soil (PT/GC; US EPA method 8240) [48]. In the P&T method, VOC's are extracted from the aqueous phase with an inert gas and transferred to a sorbent bed. Analytes trapped on the sorbent are then thermally desorbed to the column of a GC for analysis. As P&T uses an inert gas to extract analytes from water, it is free from the problems associated with solvent contamination in LLE. The intermediate sorbent trap can be eliminated if the analytes are transferred directly to the head of the GC column and cryogenically focussed. Although this decreases analysis times, a small amount of water is also transferred to the GC column which may plug the head of the column with ice so a glass-bead water trap has to be employed.

Despite their high molecular masses chlorobenzenes (including 1,2,4-trichlorobenzene) have been determined in water at part per trillion (ppt) levels by purging directly to the GC capillary column followed by cryofocussing [49-50].

P&T is also used to analyse the headspace above heated liquid and solid samples for volatile, semi-volatile, thermally stable and strongly bound compounds. This technique is recommended by the EPA as a quick screening method for contaminated soil and ground water at cleanup sites but has been shown to be quantitative and eliminates sample cleanup (HS/GC; US EPA method 3810) [48]. The major shortcoming of headspace analysis in the case of soil samples is the inability to fully extract the soil contaminants in the presence of water [51] which has lead to a lack of reproducibility [52].

P&T can achieve accurate and precise results at low detection limits [53-54], however, it is limited by expensive equipment and is prone to leaks. Traps can easily be contaminated by analytes > 200 ppb which causes sample carryover [55] and requires down time to clean the system. The desorption temperature used to remove analytes from the trap is limited by thermal stability of the analyte and sorbent material.

Even with the disadvantages described for LSE and LLE, they are still approved for the analysis of organic compounds by the US EPA [56]. Much more efficient and environmentally friendly techniques are now being employed by research teams for the extraction of organic compounds in environmental samples. These benefit from lower volumes of organic solvents whilst allowing larger sample throughput [57-59]. Reported recoveries are comparable or favourable to traditional methods of sample preparation

[60]. Their aim is ultimately to do away with the traditional methods described. New techniques notably include supercritical fluid extraction (SFE), microwave assisted extraction (MAE), accelerated solvent extraction (ASE), solid phase extraction (SPE) and solid phase microextraction (SPME). These techniques are discussed below:

2.3 Modern sample preparation techniques

2.3.1 Supercritical fluid extraction (SFE)

One of the most promising methods to recently emerge (with respect to solvent consumption) is SFE. This utilises the critical parameters of gases (or liquids) above which, they exist as 'fluids'. Fluids posses favourable extraction properties including strong solvating ability (similar to liquids) and gas-like viscosity aiding diffusion. Therefore analytes are better accessible through efficient matrix penetration and transported faster from solid matrices by enhanced solvation, thus affording extraction in a short period of time.

The presence of the solvating power of supercritical fluids in nature is evident around volcanic vents deep in the ocean where nodules of metals are thought to have been deposited by the action of supercritical water which dissolves metals and silica [61]. Supercritical water has limited application in industry due to its harsh critical parameters and its main use is in the detoxification of waste. However 'subcritical water' is becoming popular in accelerated solvent extractions (see 2.3.3).

For practical purposes supercritical CO_2 is mainly used and has found many applications in environmental science [62-67]. It has low critical parameters (temperature = 31 °C and pressure = 73 atm), is cheap, non-toxic or corrosive and non-flammable and can be used to extract liquid and solid matrices. Since the solvent strength of the fluid is related to its density, extraction enhancement can be achieved by altering the extraction pressure. Pressure can be altered for the specific extraction of certain classes of chemicals. At higher density the CO_2 becomes more polar and able to extract more polar compounds. 'Modifiers' such as methanol can also be added to the fluid to change polarity.

Similar to HPLC and SFC systems, SFE comprises a hollow stainless steel extraction cell with fittings at each end which are connected to tubes carrying the supercritical fluid through the system. Samples are placed into these vessels and fitted to the carrier tubes housed in an oven heated above the critical temperature of CO_2. A pump is used to carry the pressurised CO_2 to the extraction cell and a back pressure regulating restrictor placed at the outlet maintains pressure throughout the system. The fluid can either be continuously pumped through the cell (dynamic extraction) or the cell can be sealed at one end and the fluid used to statically extract analytes before being released from the cell in dynamic mode.

Hawthorne and Miller [68] coupled SFE (CO_2) with GC-MS to extract and analyse PAH's from samples of the National Bureau of Standards SRM 1650 (diesel exhaust particulate) and found comparable results with the certified values. Whilst Schleussinger et al. [69] found that the addition of water to soil containing PAH's facilitated their release when extracted by SFE (CO_2). Saim et al. [70] extracted a soil

contaminated with PAH's by supercritical fluid extraction, pressurised and atmospheric microwave-assisted extraction, accelerated solvent extraction and Soxhlet extraction. The recoveries of PAH's were comparable with Soxhlet except in a few cases. The advantages of the other techniques over Soxhlet were highlighted as sample mass, solvent usage and extraction time. For example comparing Soxhlet with SFE, the solvent consumption is 150 ml and 12 ml, the mass sample is 10g to 1g and the extraction time is 24 hr to 1 hr respectively.

SFE (CO_2) with sorbent trap collection and small volume solvent elution has been used to extract phenol and cresols in soil samples with high carbon content [71]. Recoveries were between 76.3 for 4-cresol and 97.9 % for 2-cresol. SFE (CO_2) has been found to be comparable to LSE for the selective isolation of organophosphates from contaminated cereals [72] and superior to LLE for the isolation of pesticides stored in freeze dried water samples [73]. Whilst LSE required preconcentration and clean-up steps and used more solvent, LLE chromatograms showed interferences from the water matrix which weren't extracted by SFE.

As with purge and trap, the extraction of aqueous samples by SFE is also a problem due to the finite solubility of water in CO_2 fluid leading to problems in chromatography when using GC. The use of SPE to extract aqueous samples followed by SFE of the SPE sorbent minimises this problem [74]. SFE has lead to many innovative experiments, including that of Barnabas et al. [75] who were able to selectively extract 'organochlorine pesticides (OCP's)' from 'phenyl urea herbicides and triazine herbicides'. The problem of excessive water carry over associated with SFE of aqueous samples was removed by first trapping a 200 ml water sample of the analytes onto (C18

Empore) SPE disks. As both classes of compounds are fully retained on these disks this not only eliminated the problem of water but also provided a solid support which could be selectively extracted by SFE. The disks were dried for 20 mins at 45 °C and inserted into an extraction cell. Extraction with CO_2 preferentially liberated OCP's, whilst re-extracting with 10 % MeOH modified CO_2 extracted the Herbicides. The extract containing OCP's was collected in hexane and analysed by GC-MS and herbicides collected in the HPLC mobile phase and analysed.

Depending on the mode of extraction SFE has advantages over LSE and LLE by its speed of extraction, elimination of the evaporation/concentrating step, reduced environmental hazard (non toxicity), suitability for thermally labile samples whilst allowing a degree of control over analyte selectivity and ease of coupling for on-line SFE-GC.

2.3.2 Microwave assisted extraction (MAE)

In MAE the solid sample and extracting solvent are contained in a sealed vessel. This sample is heated under pressure using microwave energy. The microwave power, solvent temperature and pressure can be controlled. Commercial microwave extraction devices can simultaneously extract up to 12 samples. The applicability of microwave energy to enhance extraction of polar and non-polar organic compounds from solid matrices such as soil, seeds and foodstuffs has been reported by Ganzler et al. [76]. Another advantage was the extraction of a thermally labile compound (gossypol) from cotton seed which degraded under Soxhlet conditions.

Non-polar solvents, such as toluene and hexane are not affected by microwave energy (i.e. don't heat up) which illicits molecular motion by rotation of dipoles. Therefore it is necessary to add a polar solvent in order to heat up the 'sample' or 'sample + nonpolar' solvent system, and enhance release of analytes from the matrix [77]. Water possesses a high dielectric constant and subsequently it absorbs microwave energy. Unlike Soxhlet, the system is pressurised and the solvent can be heated up above its boiling point to yield faster extractions and higher extraction recoveries. Microwave energy commonly used for scientific purposes is at a frequency of 2450 MHz. Under these conditions, alignment of molecules posessing dipoles, followed by disorder occurs 4.9×10^9 times per second, which as a result releases a lot of heat into the sample.

MAE has been seen to be more effective than Soxhlet for the extraction of polar compounds, and the efficiency was higher when the extraction solvent contained water [76]. For example, Egizabal et al. [78] reported comparable recoveries between MAE and Soxhlet for the extraction of phenols from soil. The extraction of non-polar compounds was only slightly lower than with Soxhlet. In the past the EPA has been criticized for slow adoption of new technology and in a recent publication Lopez-Avila et al. [79] researched the use of MAE in conjunction with the EPA's program for evaluating new sample preparation techniques. MAE was used to extract 6 certified reference materials containing pesticides, PAH's and phenols. The results showed comparable recoveries with Soxhlet, however both techniques failed to reach the recoveries dictated by the certified values. Soils were artificially spiked with the same chemicals as the reference materials and were extracted by MAE alongside solutions of these chemicals (used as a control). Full recovery was found from the solutions implying thermal stability and low errors due to sample handling. For the soils, recoveries were lower and of the same order

as the reference materials suggesting a strong influence by the soil matrix to irreversibly bind a finite fraction of these compounds.

Barnabas et al. [58] showed generally higher recoveries than Soxhlet for 16 PAH's in low PAH contaminated soil using MAE with the same solvent as Soxhlet (DCM), whilst for higher contaminated soils MAE extracted a higher amount of volatiles than Soxhlet (between naphthalene and anthracene) and Soxhlet gave higher recoveries for the more hydrophobic PAH's.

Due to similarity of extraction recoveries between Soxhlet and MAE found by individual research groups, the obvious advantages of MAE are reduced extraction time, solvent use and increased sample throughput. MAE also has a higher sample throughput than ASE, SFE and SPE but cannot be coupled on line with an instrument of measurement.

2.3.3 Accelerated solvent extraction (ASE)

ASE is an automated solvent extraction technique comprising a solvent reservoir and a sealed stainless steel extraction vessel similar to that used in SFE. Solid samples are weighed into the vessels and placed onto a carousel. The carousel is moved into an oven where automation seals the vessel in line with the solvent delivery system. Static extraction is generally carried out and pressure and temperature can be altered to optimise extraction efficiency.

Water at high temperatures reduces in polarity and can be used instead of traditional organic extraction solvents to extract nonpolar analytes from solid matrices. As temperature is increased, the Kow of organic compounds decreases, Henry's constants increase (see later) resulting in increased vapour pressure so they become more soluble in water. These effects coupled with waters decreased polarity at high temperatures cause rapid release of organic compounds from their sorbing matrices. For these reasons the use of hot water extraction has recently been shown to be advantageous in ASE [80].

With subcritical water extraction the loss of more volatile PAH's compared with Soxhlet wasn't found when analysing an industrial soil [81]. However a massive recovery of anthracene (324 %) was observed compared to phenanthrene (99 %) concluding that hot water/pressure conditions degraded the deuterated internal standard to d8-anthraquinone but didn't effect undeuterated anthracene. Hageman et al. [81] don't give any explanation for this selectivity and only say that hot water is relatively unreactive and wouldn't be expected to cause degradation. It may also be that at high temperature and pressure, small amounts of hydrogen peroxide and molecular hydrogen form as suggested by [82-83] via highly energetic hydrogen and hydroxy radicals, whose reactivity may account for the degradation of some organic solutes [84].

Before accelerated solvent extraction (ASE) became commercially available, research groups were using similar apparatus built 'in-house' to carry out subcritical water extractions ('ASE') on contaminated industrial soils [28, 81]. Because solid phase microextraction (see below) is well suited for the direct analysis of water samples, once the 'ASE' extraction is complete the water can either be decanted or dynamically flushed from the extraction vessel into another, prior to further extraction of the cooled liquid by

SPME. Buchholz and Pawliszyn [28] and Hageman et al. [81] analysed PAH's in a standard reference material (SRM) and used isotopically labelled analogs of the target analytes as internal standards. Smaller samples were found to yield similar reproducibilities and increased recovery (i.e. higher solution/soil ratio) compared to large samples, showing that a representative soil mass of the bulk sample can be small. Table 2.1 compares the results from three laboratories for the analysis of PAH's in a reference material supplied by The National Institute of Science and Technology (NIST urban air particulates, SRM 1649) by Soxhlet and ASE-SPME.

Table 2.1

Comparison of literature values for the extraction of PAH's from a SRM [28, 81]

	NIST values		
Method	SOXHLET	'ASE' - SPME	'ASE' - SPME
Extraction time	48 hrs	15 mins	15 mins
Extraction temperature		250 °C	250 °C
SPME fibre (thickness)		(PDMS) 100 μm	(PDMS) 30 μm
Sample weight	Not given	50 mg	50 mg
Reference	81	81	28
Concentration	μg/g	μg/g	μg/g
Fluoranthene	7.1	9.7	9.5
Pyrene	6.6	5.1	6.3
Benzo[a]pyrene	2.9	1.6	2.1

These results show that 'ASE' - SPME is comparable with Soxhlet for the determination of PAH's between different laboratories and obviously has many advantages over Soxhlet including extraction solvent type and sample through put. Comparable recoveries between laboratories using different fibre thickness also highlights the robustness of SPME.

2.3.4 Solid phase extraction (SPE)

As already mentioned, LLE is the traditional approach to the problem of extracting aqueous samples. However, the use of off-line or on-line solid phase extraction as a trace enrichment technique has been found to be an effective alternative in many applications due to its low solvent consumption and shorter handling times (1-2 hrs) [85-87].

In SPE a range of sorbents are available, tailored for selectivity and held in a cartridge or a disk. SPE is based on two processes of extraction, first a volume of water sample is eluted through the cartridge or disk and the analytes present are trapped onto the SPE sorbent. Secondly desorption of the retained compounds is via elution by a small amount of solvent or supercritical fluid or via desorption at high temperatures. As with soils, the two major retention mechanisms on solid supports are sorption and partitioning. Desorption solvents are chosen for analyte elution based on partition coefficients of the analytes of interest between sorbent and eluting solvent. Solvents used for desorption from sorbents have included hexane-diethyl ether, methanol-dichloromethane, acetone, acetone-hexane, ethanol, acetonitrile and water [88].

SPE can completely remove an analyte from its liquid matrix (i.e. exhaustive extraction) if the analytes affinity for the sorbent is high. Large volumes can be extracted to enhance trace enrichment and yield concentrations of analyte sufficient for detection and quantitation. Selection of an appropriate stationary phase can maximise extraction, but there is a limit to the volume of aqueous sample that can be extracted before elution occurs, as the analyte of interest has some capacity for the solvent it is dissolved in. This

volume depends on the capacity factor of the analyte between the sample solvent and sorbent which will be influenced by the solubility, hydrophobicity of the analyte and the mass of sorbent used (or column length). For example, Saez et al. [89] found that 1 litre of a well water sample containing triazine herbicides and their hydroxylated and dealkylated degradation products could be eluted through cartridges containing 1 g of a Sep-Pak C18-modified silica sorbent without problems of analyte breakthrough. LOD's were between 0.1 - 0.05 μg/l with UV detection. Whilst Corcia and Marchetti [90] found that 4 litres of a water sample containing the phenylurea herbicide could be eluted through a graphitised carbon black (Carbopack B) column with 10 % Fenuron lost through sample breakthrough due to its high solubility in water. The LOD for other herbicides studied was as low as 0.001 μg/l. Disks have been used for extracting and pre-concentrating pesticides, organotins and phthalates [91-94]. Immobilized liquid membranes have also been used in the disk formation for the determination of amines in aqueous samples [95]. SPE disks have large cross-sectional area allowing higher flow rates than with cartridges which is more convenient for field sampling, but due to the lower sorbent load in disks there is a lower limit to the volume of aqueous sample that can be eluted before analyte breakthrough.

An expansion on this technique can be made to accommodate more complex samples by coupling two or more cartridges in series for the pre-concentration step. This allows selective pre-concentration that can be used to remove interferences and fractionate complex mixtures. Based on the same idea, HPLC systems have been developed to incorporate on-line pre-concentration with short pre-columns containing various sorbents to replace SPE cartridges. Small glass columns packed with sorbents

have also replaced SPE cartridges to avoid contamination of the chromatograms from plasticisers often found to interfere with peaks of interest [96].

Several pre-columns can be incorporated if the sample contains compounds of widely different polarity. For example, the selective extraction of polar compounds from non-polar interferences can be achieved by coupling a non-polar pre-column acting as an interferant filter (e.g. n-alkylsilica) with a polar column to trap the target compounds of interest (i.e. styrene-divinylbenzene copolymer PRP-1). Nielen et al. [97] coupled three pre-columns in series to extract a number of solutes from waste water. A C-18 sorbent, a polymer based PRP-1 sorbent and a cation-exchange pre-column were used to trap non-polar, moderately polar and highly polar analytes. Each pre-column was subsequently analysed with reversed-phase HPLC. As with LSE and LLE, off-line extraction and preconcentration by SPE can suffer from excessive sample handling time [98].

Grob [99] developed a method specifically for the analysis of atrazine in water samples using miniaturised on-line sample enrichment with an ODS pre-column coupled to a GC-NPD. A 10 ml water sample spiked with 0.1 μg/l atrazine was eluted through the column followed by desorption with methanol-water (60:40) + 5 % 1-propanol and transfer to a GC by concurrent eluent evaporation.

Sacchero et al. [100] replaced the injection loop of a HPLC with a double trap configuration of microcolumns to achieve on-column enrichment of triazines in tap and river water followed by analysis. A LiChrospher 100 RP-18 microcolumn was used to trap lipophilic interferences whilst a coupled supelclean Envi-18 microcolumn was used to enrich the triazine herbicides present in the water. Similarly, an anion exchange guard

column (20 μl) installed in place of the sample loop on the injection valve of a HPLC has been used to retain (-)-malic acid from fruit juices whilst removing sugar interferences which co-elute with (-)-malic acid [101].

The limitations of SPE include low recoveries due to sample breakthrough at high concentrations or volumes of compounds with low partition coefficients in the selected sorbent [96]. The SPE cartridges and in particular the discs are prone to clogging with samples containing large amounts of sediment. Silica bonded octadecyl is often preferred for the removal of several types of pesticides from water, however this ability also removes interferences from the matrix [102] which can be pre-concentrated giving rise to high background and co-elution [103]. Matrix effects can be minimised by connection of several cartridges or pre-columns to fractionate the sample, whilst breakthrough can be minimised through knowledge of the capacity factors of the analytes.

Preconcentration and analysis using pre-columns on-line with HPLC or GC has the advantage over off-line SPE with cartridges of giving higher reproducibility and sensitivity. There is also the possibility of automation which would increase sample throughput.

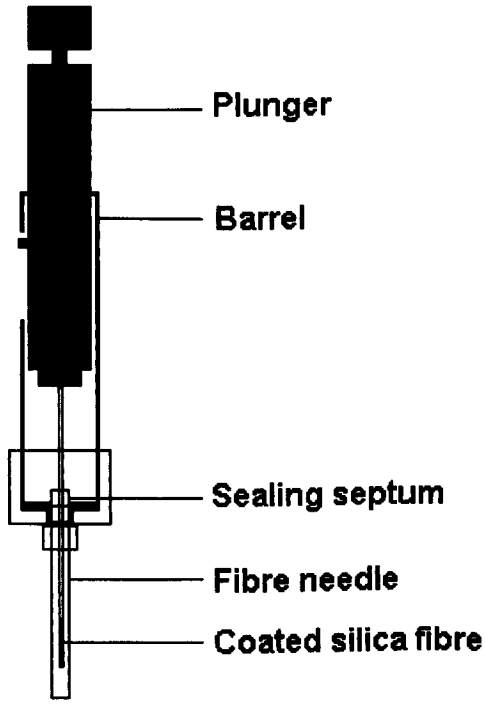
2.3.5 Solid phase microextraction (SPME)

SPME, pioneered by Prof. Janusz Pawliszyn, is the most environmentally friendly extraction technique to recently emerge and is gaining recognition as a solvent-free alternative for sample preparation and is an excellent tool for pre-concentration of pollutants in air, water and soils. In SPME the bottom centimetre of a small diameter

optical fibre made of chemically inert fused silica is coated with either a solid (i.e. polyacrylate 'PA') or liquid (i.e. polydimethylsiloxane 'PDMS') organic polymer [104-105]. The stationary phase coating replaces the solvent in LLE and the sorbent packing in SPE. The fibre is glued into a length of stainless steel tubing which runs up through a syringe needle attached to a plunger. The plunger exposes and retracts the fibre through the syringe needle. The SPME fibre assembly is shown in figure 2.1.

Figure 2.1

Diagram of the SPME fibre assembly



The needle has 3 main purposes :

- It protects the fibre from external interference (both physical and chemical).

- It is used to penetrate the sample vial septum and GC port septum, and therefore allows simple introduction to the sample matrix for partitioning and introduction into the GC for thermal desorption.

- It avoids excessive desorption of more volatile analytes between the end of extraction and introduction into the GC.

The two main methods of extraction in SPME are 'direct' analysis and 'headspace' analysis. In direct analysis the fibre is exposed directly to a liquid sample or soil slurry and the analytes in the sample partition between the solution and the fibre. In headspace analysis the fibre is exposed to the air above a solid or liquid sample and as with direct extraction, the analytes in the headspace are partitioned between the headspace and the fibre. The degree of partitioning depends on the properties of the analyte and the fibre. Both these sampling techniques, the factors governing partitioning, and methods for enhancing extraction will be discussed in detail in 'Part B' (chapters 7-10).

The evolution in SPME method development and applications has progressed rapidly since its introduction in 1989 by Prof. Januz Pawliszyn, who has released the most informative literature on the subject to date [26, 106]. Some of the first experiments involving SPME analysed benzene, toluene, ethylbenzene, and the xylene isomers

(BTEX) in water [27], sand, clay and sludge [1] and more recently in waste water [107]. Other applications have included the extraction of S-triazine herbicides from water [108], nitrogen containing herbicides and chlorinated pesticides from water [109], melted arctic snow and orange juice [110]. PAH's have been determined in contaminated sewage, by headspace SPME [111] and PAH's and PCB's determined in water [112]. Hetero aromatic compounds with N, S or O in the aromatic ring system found in creosote-contaminated groundwater have also been analysed by SPME, with GC-MS [113]. A correlation was found between K_{PDMS} and Kow for nonpolar compounds but not with phenols. As PDMS is a relatively nonpolar liquid type coating these results are in agreement with the partitioning mechanism for nonpolar compounds and stronger H-bonding mechanism for polar compounds.

Moens et al. [114] used SPME to develop a method for the simultaneous extraction and derivatization, of organomercury, tin, and lead compounds from the matrix and analysed the extracts by GC coupled with inductively coupled plasma mass spectrometry detection. Poerschmann et al. [115] have applied a similar method to investigate the sorption behaviour of ionic and non-ionic alkylated tin compounds onto humic matter.

Surface derivatisation of the fibers silanol groups with trimethylsilylchloride (to further lower the polarity) has been found to increase the extraction of PAH's directly from a soil methanol slurry [116]. Headspace SPME can be applied to extract VOC's physically sorbed to solid samples. However, for non volatiles or strongly bound compounds the low concentration in the headspace will limit partitioning. Increased sensitivity can be achieved by elevating the temperature to over come the energy of

desorption (Edes*) limitations and speeding up mass transport into the headspace by increasing the vapour pressure of the analytes. Zhang and Pawliszyn [1] found that adding 10-30 % water to contaminated sand facilitated release into the headspace further on heating. The most common fibres employed are PDMS and PA of differing coating thickness. Recently other fibre coatings have been employed for specific applications including the use of extra fine powdered activated charcoal to extract BTEX compounds from the headspace [117] whilst a more polar carbowax divinyl benzene fibre has been used to extract derivatised amines [118]. Both fibres enhanced extraction when compared with PDMS. Derivatisation generally makes a compound more nonpolar and/or volatile therefore increasing the partition coefficient into the fibre and has been shown to enhance sensitivity for analysing amines in air and water by SPME [118]. Derivatisation also decreases the tailing of peaks frequently associated with the chromatography of polar compounds.

Phenols have been extracted by SPME using headspace and direct extraction [28, 119-120]. In these extractions the water matrix is commonly acidified (to maintain the neutrality of the phenols) and saturated with salt (to decrease solubility) to increase recovery. It has been suggested that the high correlation found between partitioning values of phenols into the polyacrylate fibre compared with their Kow's indicated that SPME is a viable method for estimating log Kow values < 3.5 [120]. However, as different physiochemical and quantum descriptors contribute towards the K_{SPME} value, different relationships will be valid for different classes of chemicals. This has been shown by Pawlizsyn [121] and Vaes et al. [122] for polar chemicals which have higher K_{SPME} than nonpolar chemicals with the same hydrophobicity, probably because the H-bonding physiochemical descriptor increases their interaction with the fibre coating.

Modifications of the K_{SPME} value to account for such descriptors (whether fibre or molecule dependent) may increase the correlation with Kow for polar compounds as has been the case in studies with sorption to organic matter from different soils where an aromatic descriptor is mentioned (see chapter 6.0).

The advantages of SPME found in the above literature, are summarised below:

- Selective extraction of polar and non-polar, volatile and non-volatile organic compounds from gas, liquid and solid matrices.

- Sensitivity (detection limits ppb-ppt) with a wide linear concentration range.

- Portability and compatibility with any GC equipped with split/splitless or on column injection modes.

- Combination of simple inexpensive apparatus with short sampling times

- Solvent free and therefore economic due to the elimination of solvent purchase and disposal costs and fibres are reusable up to 200 times.

2.4 Summary and conclusions

Although MAE, SFE and ASE have shorter extraction times and smaller solvent and sample volumes compared to Soxhlet (from 24 hr for Soxhlet to 20 min for MAE) none of these methods can easily be coupled to GC for fully automated analysis. SPME

can be coupled to GC and automated to extract, pre-concentrate and analyse samples in a single step. As a result of solventless injection the continued efficiency of the GC is maintained and the chromatography is enhanced [105].

Unlike SPE, SPME doesn't require exhaustive removal of organic compounds from large volumes of water. (unless analytes have very high partition coefficients). SPME can incorporate hot water extraction step (for liquids or solids) on a smaller scale, which may become automated and coupled to separation and detection systems in future applications. More novel applications involving SPME will be discussed in chapters 8.0 and 10.0.

2.5 References

1. Z. Zhang and J. Pawliszyn, *J. High Resolut. Chrom.*, **16** (1993) 689.
2. B. Macgillivray and J. Pawliszyn, *J. Chromatogr. Sci.*, **32** (1994) 317.
3. G.W. Bailey and J.L. White, *J. Agric. Food Chem.*, **12** (1964) 324.
4. W.P. Ball and P.V. Roberts, In '*Organic substances and sediments in water*'; Lewis Publishers Inc: Chelsea, MI, Impress. **Chap. 13** (1991) 273.
5. J. Porschmann, F.D. Kopinke, M. Remmler, K. Mackenzie, W. Geyer and S. Mothes, *J. Chromatogr. A*, **750** (1996) 287.
6. M.A. Callahan and M.W. Slimak, Water related environmental fate of 129 priority pollutants, EPA-440/4-79-029b; US Environmental Protection Agency. Washington DC. (1979) **VOL II.**
7. M.S. Tswett, *J. Chem. Educ.*, **238** (1967) 44.
8. M.S. Tswett, *Ber. Dtsch. Bot. Ges.*, **24** (1906) 235.

9. L.S. Lee, P. Suresh, C. Rao, P. Nkadi-Kizza, and J.J. Delfino, *Environ. Sci. Technol.*, **24** (1990) 654.

10. R.L. Gustafson and J. Paleos, S.D. Faust and J.V. Hunter, In *'Organic compounds in the aquatic environment'*; Marcel Dekker Inc., New York, **chap.10** (1971) 213.

11. D.C. Kennedy, *Ind. Eng. Chem. Prod. Res. Dev.*, **12** (1973) 56.

12. J. Rosenbaum, *Trans. Amer. Soc. Artificial Organs.*, **16** (1970) 134.

13. J. Rosenbaum, *Clin. Toxicol.*, **5** (1972) 331.

14. J. Paleos, *Journal of Colloid and Interface Science,* **31** (1969) 7.

15. D.C. Kennedy, *Environ. Sci. Technol.*, **7** (1973) 138.

16. B.W. Stevans and J.W. Kerner, *Chem. Eng.*, **82** (1975) 84.

17. R. Kunin, *Pure Appl. Chem.*, **46** (1976) 205.

18. G. Font, J. Manes, J.C. Molto and Y. Pico, *J. Chromatogr.*, **642** (1993) 135.

19. W.W. Wood, T.F. Kraemer and P.P. Hearn, Jr., *Science,* **247** (1990) 1569.

20. M.L. Brusseau, R.E. Jessup, P. Suresh and C. Rao, *Environ. Sci. Technol.*, **25** (1991) 134.

21. M. Alexander, *Environ. Sci. Technol.*, **29** (1995) 2713.

22. J.J. Pignatello and B. Xing, *Environ. Sci. Technol.*, **30** (1996) 1.

23. P.B. Hatzinger and M. Alexander, *Environ. Sci. Technol.*, **29** (1995) 537.

24. L.H. Keith and W.A. Telliard, *Environ. Sci. Technol.*, **13** (1979) 416.

25. M.C. Hennion, *Trends in Anal. Chem.*, **10** (1991) 317.

26. Z. Zhang, M.J. Yang and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 844.

27. C.L. Arthur, L.M. Killam, S. Motlagh, M. Lim, D.W. Potter and J. Pawliszyn, *Environ. Sci. Technol.*, **26** (1992) 979.

28. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 160.

29. British Gas Method '*Polynuclear Aromatic Hydrocarbons in soils by GCMS*'; issued for use by Northumbrian water (Analytical and Environmental Services) by S.P.Owen, Method no. **O27** (1993) 1.

30. B.G. Oliver and K.D. Bothen, *Intern. J. Environ. Anal. Chem.*, **12** (1982) 131.

31. C.S. Helling, W. Zhuang, T.J. Gish, C.B. Coffman, A.R. Isensee, P.C. Kearney, D.R. Hoagland and M.D. Woodwarrd, *Chemosphere*, **17** (1988) 175.

32. T. Harner and T.F. Bidleman, *Environ. Sci. Technol.*, **32** (1998) 1494.

33. J.H. Clemons, L.M. Allan, C.H. Marvin, Z. Wu, B.E. McCarry, D.W. Bryant and T.R. Zacharewski, *Environ. Sci. Technol.*, **32** (1998) 1853.

34. H. Roseboom and H.A. Herbold, *J. Chromatogr.*, **202** (1980) 431.

35. M.L. Lee, M.V. Novotny and K.D. Bartle, *In 'Analytical Chemistry Of Polycyclic Aromatic Compounds'*; Academic Press, New York, (1981) 78.

36. A.J. Nunez, L.F. Gonzalez and J. Janak, *J. Chromatogr.*, **300** (1984) 127.

37. C.F. Poole and S.A. Schuette, *HRC & CC*, **6** (1983) 526.

38. L.D. Johnson and R.G. Merrill, *Toxicol. Environ. Chem. Rev.*, **6** (1983) 109.

39. W.H. Griest, L.B. Yeats, Jr., and J.E. Calon, *Anal. Chem* ., **52** (1980) 199.

40. C.L. Arthur, D.W. Potter, K.D. Buchholz, S. Motlagh and J. Pawliszyn, *LC- GC*, **10** (1992) 656.

41. C.E. McKone, T.H. Byast and R.J. Hance, *Analyst*, **97** (1972) 653.

42. H.B. Lee and Y.D. Stokker, *J. Assoc. Off. Anal. Chem.*, **69** (1986) 568.

43. M. Guidotti, *J. High Resolut. Chromatogr.*, **19** (1996) 469.

44. C. Samson, V.N. Mallet, J. Doull and G. Brun, *Intern. J. Environ. Anal. Chem.*, **64** (1996) 111.

45. SPME. Supelco Application notes: **58** and **83** (1994).

46. M.D. Erickson, M.T. Giguere and D.A. Whitaker, *Anal. Lett.*, **14(A11)** (1981) 841.

47. T.A. Bellar and J.J. Lichtenberg, *J. Am. Water Works Assoc.*, **66** (1974) 739.

48. US Environmental Protection Agency, Test Methods for Evaluating Solid Waste, SW-846, 3rd Ed.; EPA/OSWER, Washington DC, (1986).

49. R. Borrelli, T. Fiorani and P. Golfetto, *J. High Resolut. Chromatogr.*, **19** (1996) 457.

50. S.A. Rounds and J.F. Pankow, *J. Chomatogr.*, **629** (1993) 321.

51. S.K. Poole and C.F. Poole, *Anal. Comm.*, **33** (1996) 417.

52. S.G. Pavlostathis and G.N. Mathavan, *Environ. Technol.*, **13** (1992) 23.

53. M.R. Driss and M.L. Bouguerra, *Int. J. Environ. Anal. Chem.*, **45** (1991) 193.

54. R. Otson and C. Chan, *Int. J. Anal. Chem.*, **30** (1987) 275.

55. R. Westendorf and H. Lehan, '*An Evaluation of the problem of carry over in Purge and Trap GC*'; Tekmar Company, Cincinnati, OH. Cross reference [2] (1994).

56. L.S. Clesceri, A.E. Greengerg and R.R. Trussell, Eds.; '*Standard methods for the examination of water and wastewater*'; 17th Edn.; American Public Health Association: Washington DC, 1989.

57. S. Popilloud and W. Haerdi, *Chromatographia,* **38** (1994) 514.

58. I.J. Barnabas, J.R. Dean, I.A. Fowlis and S.P. Owen, *Analyst,* **120** (1995) 1897.

59. I.J. Barnabas, J.R. Dean, S.M. Hitchin and S.P. Owen, *Anal. Chem.*, **66** (1994) 844.

60. J.R. Dean, I.J. Barnabas and I.A. Fowlis, *Anal. Proc. Inc. Anal. Comm.*, **32** (1995) 305.

61. M.R. Andersen, J.T. Swanson, N.L. Porter and B.E. Richter, *J. Chromatogr. Sci.*, **27** (1989) 371.

62. W.C. Koskinen, H.H. Cheng, L.J. Jarvis and B.A. Sorenson, *Intern. J. Environ. Anal. Chem.*, **58** (1995) 379.

63. C. Lutge, I. Reiβ, A. Schleuβinger and S. Schulz, *The Journal of Supercritical fluids*, **7** (1994) 265.

64. S. Ciron, S. Dupas, P. Scribe and D. Barcelo, *J. Chromatogr. A*, **665** (1994) 295.

65. L.Q. Xie, K.E. Markides and M.L. Lee, *Anal. Biochem.*, **200** (1992) 7.

66. K.D. Bartle, T. Boddington, A.A. Clifford and N.J. Cotton, *Anal. Chem.*, **63** (1991) 2371.

67. E.G. van der Velde, M. Dietvorst, C.P. Swart, M.R. Ramlal and P.R. Kootstra, *J. Chromatogr. A*, **683** (1994) 167.

68. S.B. Hawthorne and D.J. Miller, *J. Chromatogr. Sci.*, **24** (1986) 258.

69. A. Schleussinger, B. Ohlmeirer, I. Reiss and S. Schulz, *Environ. Sci. Technol.*, **30** (1996) 3199.

70. N. Saim, J.R. Dean, M.P. Abdullah and Z. Zakaria, *J. Chromatogr. A.*, **791** (1997) 361.

71. M.P. Llompart, R.A. Lorenzo and R. Cela, *J. Chromatogr. A*, **723** (1996) 123.

72. J. Poulstka, K. Holadova and J. Hajslova, *Intern. J. Environ. Anal. Chem.*, **60** (1995) 139.

73. R. Alzaga, G. Durand, D. Barcelo and J.M. Bayona, *Chromatographia*, **38** (1994) 502.

74. R. Alzaga and J.M. Bayona, *J. Chromatogr.*, **655** (1993) 51.

75. I.J. Barnabas, J.R. Dean and S.M. Hitchen, *J. Chromatogr. Sci.*, **32** (1994) 547.

76. K. Ganzler, A. Salgo and K. Valko, *J. Chromatogr.*, **371** (1986) 299.

77. F.I. Onsuka and K.A. Terry, *Chromatogrphia*, **36** (1993) 191.

78. A. Egizabal, O. Zuloaga, N. Etxebarria, L.A. Fernandez and J.M. Madariaga, *Analyst*, **123** (1998) 1679.

79. V.Lopez-Avila, R. Young and W.F. Berkert, *Anal. Chem.*, **66** (1994) 1097.

80. Y. Wang, M. Bonilla and H.M. McNair, *J. High Resolut. Chromatogr.*, **20** (1997) 213.

81. K.J. Hageman, L. Mazeas, C.B. Grabanski, D.J. Miller and S.B. Hawthorne, *Anal. Chem.*, **68** (1996) 3892.

82. M.A. Khenokh and E.M. Lapinskaya, *J. Gen. Chem.*, **26** (1956) 2727.

83. K.S. Suslick, Ultrasound: Its Chemical, Physical and Biological effects, VCH, New York (1988).

84. A.P.P. Silva, S.K. Laughlin, S.J. Weeks and W.H. Buttermore, *Polyaromatic Compounds*, **1** (1990) 125.

85. G.A. Junk and J.J. Richard, *Anal. Chem.*, **60** (1988) 451.

86. E. Chladek and R.S. Marano, *J. Chromatogr. Sci.*, **22** (1984) 313.

87. S. Fingler, V. Drevenkar and Z. Vasilic, *Mikrochim. Acta*, **2** (1987)163.

88. G. Font, J. Manes, J.C. Molto and Y. Pico, *J. Chromatogr.*, **642** (1993) 135,

89. A. Saez, D. Gomez de Barreda, M. Gamon, J.G. de la Cuadra, E. Lorenzo and C. Peris, *J. Chromatogr. A*, **721** (1996) 107.

90. A.D. Corcia and M. Marchetti, *J. Chromatogr.*, **541** (1991) 365.

91. G. Durand and D. Barcelo, *Talanta*, **40** (1993) 1665.

92. D.F. Hagen, C.G. Markell, G.A. Schmitt and D.D. Blevins, *Anal. Chim. Acta*, **236** (1990) 157.

93. O. Evans, B.J. Jacobs and A.L. Cohen, *Analyst*, **116** (1991) 15.

94. A. Kraut-Vass and J. Thoma, *J. Chromatogr.*, **538** (1991) 233.

95. G. Nilve and R. Stebbins, *Chromatographia*, **32** (1991) 269.

96. J.C. Molto, Y. Pico, G. Font and J. Manes, *J. Chromatogr.*, **555** (1991) 137.

97. M.W.F. Neilen, U.A.Th. Brinkmana and R.W. Frei, *Anal. Chem.*, **37** (1989) 45.

98. M. Battista, A.D. Corcia and M. Marchetti, *Anal. Chem.*, **61** (1989) 935.

99. C. Grob, *J. Chromatogr.*, **473** (1989) 423.

100. G. Sacchero, C. Sarzanini and E. Mentasti, *J. Chromatogr. A*, **671** (1994) 151

101. D.M. Goodall and Z. Wu, *Anal. Proc.*, **29** (1992) 238.

102. C.C.J. Land, *LC-GC INT.*, **7** (1994) 215.

103. V. Coquart and M.-C. Hennion, *J. Chromatogr.*, **553** (1991) 329.

104. D. Louch, S. Motlagh and J. Pawliszyn, *Anal. Chem.*, **64** (1992) 1187.

105. C.L. Arthur, M. Chai and J. Pawliszyn, *J. Microcol. Sep.*, **5** (1993) 51.

106. C.L. Arthur, R. Belardi, K. Pratt, S. Motlagh and J. Pawliszyn
J. High Resolut. Chromatogr., **15** (1992) 741.

107. I. Valor and C. Cortada, *J. High Resolut. Chromatogr.*, **19** (1996) 472.

108. I.J. Barnabas, J.R. Dean, I.A. Fowlis and S.P. Owen, *J. Chromatogr. A*, **705** (1995) 305.

109. A.A. Boyd-Boland and J. Pawliszyn, *J. Chromatogr. A*, **704** (1995) 163.

110. A.A. Boyd-Boland, S. Magdic and J. Pawliszyn, *Analyst*, **121** (1996) 929.

111. Z. Zhang and J. Pawliszyn, *Anal.Chem.*, **65** (1993) 1843.

112. D.W. Potter and J. Pawliszyn, *Environ. Sci. Technol.*, **28** (1994) 298.

113. S.S. Johansen and J.Pawliszyn, *J.High Resolut. Chromatogr.*, **19** (1996) 627.

114. L. Moens, T.D. Smaele and R. Dams, *Anal. Chem.*, **69** (1997) 1604.

115. J. Poerschmann, F.D. Kopinke and J. Pawliszyn, *Environ. Sci. Technol.*, **31** (1997) 3629.

116. M.E. Cisper, W.L. Earl, N.S. Nogar and P.H. Hemberger, *Anal. Chem.*, **66** (1994) 1897.

117. D. Djozan and Y. Assadi, *Chromatographia,* **45** (1997) 183.

118. L. Pan, J.M. Chong and J. Pawliszyn, *J. Chromatogr. A,* **773** (1997) 249.

119. Z. Penton, SPME Varian Application Note **11**, (1996).

120. J.R. Dean , W.R. Tomlinson, V. Makovskaya, R. Cumming, M. Hetheridge and M. Comber, *Anal. Chem.,* **68** (1996) 130.

121. J. Pawliszyn, In *'Solid Phase Microextraction, Theory and Practice'*; Wiley-VCH, Inc. John Wiley & Sons, Inc., 605 Third Avenue, New York, NY (1997).

122. W.H.J. Vaes, C. Hamwijk, E.U. Ramos, H.J.M. Verhaar and J.L.M. Hermens, *Anal. Chem.,* **68** (1996) 4458.

Chapter 3.0

Experimental instrumentation and reagents

3.1 Soil characterisation techniques

3.1.1 Reagents

The following reagents were obtained from BDH AnalaR, Merck, (Poole, Dorset, UK) for use in soil characterisation studies. H_2SO_4, $K_2Cr_2O_7$, HCl, $CaCl_2$, pH 4 and pH 7 buffers, $BaCl_2$, $MgSO_4$, Triethanolamine, EDTA - disodium salt, NH_4OH and the indicator - Omega chrome black. Soils were collected from locations given in chapter 4.

3.1.2 Apparatus

A GallenKamp muffle furnace oven (Fisher Scientific, Loughborough, UK) was used for combustion in the experiments involving the determination of organic matter content. A Cecil 2000 series C2030 UV spectrophotometer (Scientific & Medical Products Ltd., Shirley Institute, Manchester, UK) was used in the determination of organic carbon content. A drying oven obtained from Graseby Specac Ltd. (Orpington, Kent, UK) was used for drying in determining moisture content and water holding capacity. Soil acidity was measured with a Jenway 3010 pH meter (Fisher Scientific, Loughborough, UK).

3.2 Shake flask extraction

3.2.1 Reagents

The methanol and deionised water used, were HPLC grade. Celite (Celite for GLC) was obtained from BDH AnalaR, Merck, (Poole, Dorset, UK). Soils were collected from locations given in chapter 4. Sand was supplied by the University of Northumbria at Newcastle. The following phenols were 99 + % analytical grade obtained from Aldrich Chemical Co., (Gillingham, Dorset, UK) for use in shake flask studies. Fig. 3.1 (Phenol), fig. 3.2 (93-methylphenol), fig. 3.3 (4-methylphenol), fig. 3.4 (4-ethylphenol), fig. 3.5 (1-naphthol) and fig. 3.6 (2-naphthol).

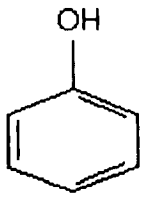


Fig. 3.1

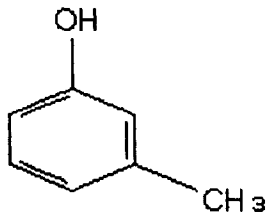


Fig. 3.2

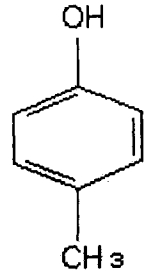


Fig. 3.3

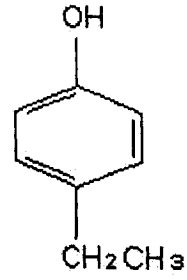
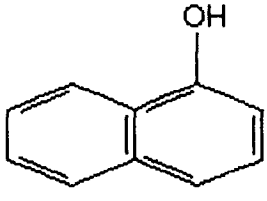


Fig. 3.4



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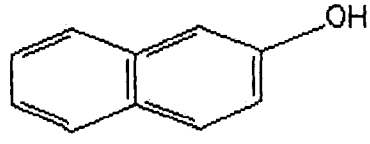


Fig. 3.6

3.2.2 Apparatus

High performance liquid chromatography was used to separate phenols and an ultra violet-visible detector was used for quantification at a fixed wavelength of 275 nm. The HPLC pump was a Gilson 305 (Midleton, WI, USA) which pumped 40 % ACN, 59 % H_2O and 1 % acetic acid at 1 ml/min. The detector was a Jasco UV VIS-975 (Tokyo, Japan) and the integrator was a Spectra physics SP4200 (Fermont, CA, USA). The column oven used was a Jones Chromatography 7970 block heater obtained from Fisher Scientific (Loughborough, UK) and set at 30 °C. The stationary phase column used was an ODS2, 25 cm long, 4. 6 mm i.d. from Phase Separations Ltd., (Deeside, Flintshire, UK).

Samples were injected into the HPLC system via a Rheodyne valve fitted with 20 µl injection loop. Shake flask extractions were carried out using a rotating disk (Warburg mixer) an 100 ml screw top glass flasks fitted with Teflon lined caps. All soil water samples were filtered through 0.45 µm membrane acrodisk filters (obtained from Phase Separations Ltd., Deeside, Flintshire.UK) to remove any suspended matter.

3.3 Batch shake flask equilibration

3.3.1 Reagents

The radiolabelled 1-naphthol-1-14 R and phenol-1-14 R (obtained from Sigma-Aldrich Company Ltd., Poole, Dorset. UK) used for partitioning investigations had specific radioactivity levels of 0.285 x 10^6 Bq/µmol and 1.443 x 10^6 Bq/µmol

respectively, and a radiochemical purity of 96.6 %. Inactive naphthol and phenol were 99 + % analytical grade obtained from Aldrich Chemical Co. (Gillingham, Dorset., UK). Liquid Scintillation cocktail (Ultima Gold) was obtained from Packard Instrument Company (Pangbourne, Berkshire, UK).

3.3.2 Apparatus

Radioactive samples were added to Ultima Gold liquid scintillation cocktail and counted using a Packard Tri-Carb 2000CA liquid scintillation Packard Instrument Company, Pangbourne, Berkshire, UK). All soil water samples were filtered through 0.45 μm membrane acrodisk filters (obtained from Phase Separations Ltd., Deeside, Flintshire.UK) to remove any suspended matter.

3.4 Solid phase microextraction of water samples

3.4.1 Reagents

Acetone and distilled water were HPLC grade. The chlorobenzenes used in these studies were 99 + % analytical grade from Aldrich Chemical Co. (Gillingham, Dorset, UK). The structures of the chlorobenzenes used are shown in fig. 3.7 (1,3,5-trichlorobenzene), fig. 3.8 (1,2,4-trichlorobenzene), fig. 3.9 (1,2,3-trichlorobenzene), fig. 3.10 (1,2,4,5-tertrachlorobenzene) and fig. 3.11 (pentachlorobenzene).

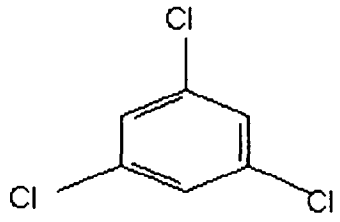


Fig. 3.7

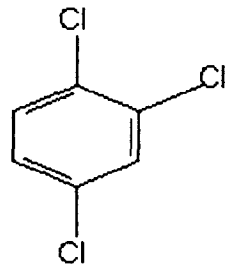


Fig. 3.8

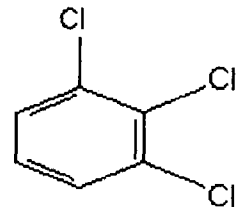


Fig. 3.9

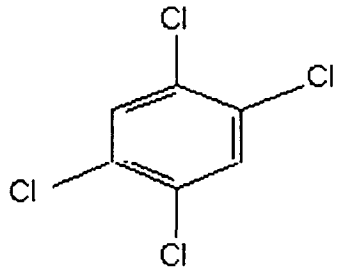


Fig. 3.10

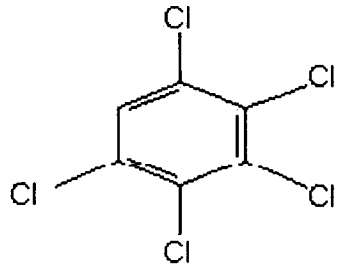


Fig. 3.11

3.4.2 Apparatus

A solid phase microextraction Varian 8100 Autosampler was coupled with an SPME Pioneer GC077 Varian Star Chromatography Workstation (Varian Ltd. Surrey) to analyse water samples. 85 μm polyacrylate and 100 μm polydimethylsiloxane fibres were obtained from Supelco (Poole, Dorset, UK). These fibres were conditioned in the injection port according to the manufacturers conditions prior to use. Samples were extracted from 2 ml crimp top or screw top autosampler vials obtained from Phase Separations Ltd. (Deeside, Flintshire, UK).

Gas chromatography was also employed using a Varian Star 3400GC (Varian Ltd., Surrey, UK) with electron capture detection (GC-ECD) with a 15 mCi ^{63}Ni source. The column used for separation was a HP-5MS crosslinked 5 % PhMe Silicone (30 m

length x 0.25 mm i.d. x 0.25 μm film thickness) from Crawford Scientific (Strathaven, UK). Thermogreen injection septa (Supelco, Poole, Dorset) were used as a gas seal in the injection port. For the chromatographic conditions, the detector temperature was set at 300 °C whilst the injector temperature was set at 280 °C when polyacrylate fibres were used and at 250 °C when polydimethylsiloxane fibres were used. The optimised GC separation conditions for chlorobenzenes used an initial column temperature set at 50 °C and held for 10 mins during desorption. The split valve was opened after 11 mins and the column oven rose in temperature to 285 °C at 10 °C / min. The helium split flow was 50 ml/min with the hydrogen carrier gas at 15 psi.

3.5 Solid phase microextraction of solid samples

3.5.1 Reagents and reference materials

DCM, Acetone and distilled water were all HPLC grade. All chlorobenzenes and PAH's were 99 + % analytical grade from Aldrich Chemical Co. (Gillingham, Dorset, UK). The structures of the PAH's used in these studies are shown in fig. 3.12 (naphthalene), fig. 3.13 (1-methylnaphthalene), fig. 3.14 (fluorene), fig. 3.15 (phenanthrene), fig. 3.16 (anthracene), fig. 3.17 (fluoranthene) and fig. 3.18 (pyrene).

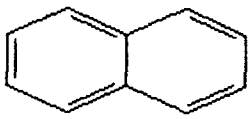


Fig. 3.11

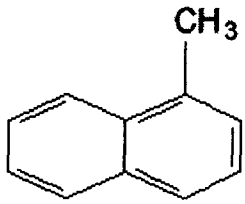


Fig. 3.12

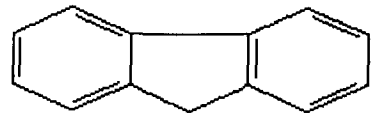


Fig. 3.13

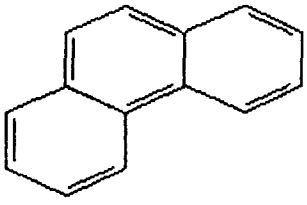


Fig. 3.14

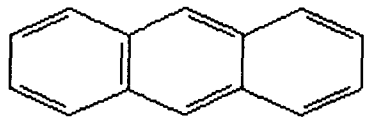


Fig. 3.15

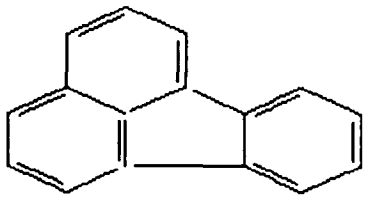


Fig. 3.16

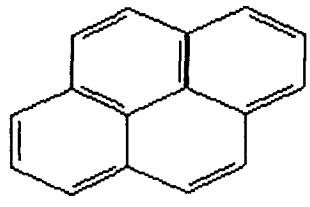


Fig. 3.17

Certified Reference Materials of contaminated soils were obtained for use in these investigations from the '*European Commission, Community Bureau of Reference (BCR)*' and the '*Laboratory of the Government Chemist (LGC)*'. Reference Material BCR No. 529 was a contaminated sandy soil. Reference Material No. 530 was a contaminated clay soil. Reference Material LGC 6138 was a contaminated coal-carbonisation site soil. The BCR soils were contaminated with chlorophenols and chlorobenzenes whilst the LGC soil was contaminated with PAH's and also had a high aluminium, iron and sulphur content. Three industrially contaminated soils ('contaminated soil 1', 'contaminated soil 2' and 'contaminated soil 3') were supplied for analysis by Northumbrian water (Howdon, Northumberland, UK). Diesel soot was obtained for analysis from the exhaust of a diesel vehicle.

3.5.2 Apparatus

A manual solid phase microextraction holder was used for these experiments and 85 μm polyacrylate or 100 μm polydimethylsiloxane fibres (obtained from Supelco, Poole, Dorset) were conditioned in the injection port of the GC according to the manufacturers conditions prior to use. Samples were extracted from 2 ml crimp top or screw top autosampler vials or 10 ml capless sample vials obtained from Phase Separations Ltd. (Deeside, Flintshire, UK).

Gas chromatography with mass spectrometry detection was carried out on a Hewlett Packard G1800A GCD system with Electron Ionisation Detector and a HP Vectro 486 data system (all obtained from Hewlet Packard, Godalming, Suurey, UK). The chromatographic column used was a DB-1 Dimethylpolysiloxane (30 m length x 0.25 mm i.d. x 0.25 μm film thickness) from Jones Chromatography (J&W Scientific, Hengoed, Mid. Glam., UK). Thermogreen injection septa (Supelco, Poole, Dorset) were used as a gas seal in the injection port.

Accelerated solvent extractions (ASE) were carried out using an ASE 200 accelerated solvent extractor obtained from Dionex (Camberley, Surrey, UK). Soxhlet extractions employed refluxing apparatus supplied by the University of Northumbria at Newcastle.

For the chromatographic conditions, the detector temperature was set at 300 °C whilst the injector temperature was set at 280 °C when polyacrylate fibres were used and at 250 °C when polydimethylsiloxane fibres were used. The optimised GC separation

conditions for chlorobenzenes and PAH's used an initial column temperature set at 50 °C and held for 10 mins during desorption. The split valve was opened after 11 mins and the column oven rose in temperature to 285 °C at 10 °C / min. The helium split flow was 50 ml/min with the hydrogen carrier gas at 15 psi.

Part A

The fate of phenolic compounds in the environment

A series of experiments were undertaken to measure and understand the mechanisms of sorption and partitioning of phenols in soil. Soil was chosen as an environmental matrix in our studies as it is considered to be the major barrier between the release of a pollutant and its subsequent contact with plants and animals. Understanding the processes governing sorption and partitioning of phenols in soils is therefore a worthwhile exercise to be able to predict their fate in the environment.

Chapter 4.0

Soil collection and characterisation

4.1 Field excursion - soil sampling (19/4/96)

Soil samples were collected from 4 contrasting locations to represent a variety of environmental characteristics. Podzols and Rendzinas are frequently situated in the European Community [1] and are represented here by sites 1 and 4, respectively. Samples at each site were taken from several depths to reflect the geomorphological horizons of the soil profile. Each soil was air dried and homogenised to pass through a 2 mm sieve to obtain a constant maximum grain size whilst removing large pieces of roots and pebbles. Further treatment was avoided to minimise changes in the soils natural sorption properties.

Site 1 Thorngrafton Common, near Bardon Mill (GR - 783665) OS Sheet 87, Hexham and Haltwhistle. Humus Iron Podzol (Anglezarke Series) on Heather moorland, 10^{o} slope with south-facing aspect, carboniferous sandstone with thin loamy drift cover.

- **Site 1 H/Ah** (0 - 7 cm) was a black (5YR 2.5/1) peaty with abundant roots and bleached quartz grains, sharp lower boundary.
- **Site 1 Ea** (7 - 20 cm) was a dark greyish brown (10YR 4/2) bleached horizon with sandy texture, sharp lower boundary.
- **Site 1 Bhs** (20 - 35 cm) was a strong brown (7.5YR 4/6) humus and sesquioxide enriched horizon with thin convoluted iron pan, indurated and stony.
- **Site 1 C** (> 35 cm) was a brownish yellow (10YR 6/6) fine sandy drift parent material.

Site 2 Muckle Moss, near Bardon Mill (GR - 802665) OS Sheet 87 as above. Gleyed brown earth (Rivington-Nercwys Series) on good quality (improved) pasture, 4° slope with south-facing aspect, loamy drift from carboniferous sandstone.

- **Site 2 Ah** (0 - 13 cm) was a black (10YR 2/1) humus rich topsoil, intimately mixed with mineral material, abundant roots, a few stones and earthworms present, with a merging lower boundary.
- **Site 2 Bg** (13 - 25 cm) was a dark brown (10YR 3/3) slightly mottled loamy sand, with a merging lower boundary.
- **Site 2 BCg** (>25 cm) was a pale brown (10YR 6/3) to strong brown (7.5YR 5/6) mottled loamy sand with a few stones.

Site 3 Muckle Moss, near Bardon Mill (GR - 798668). OS Sheet 87 as above. Fibrous peat (Winter Hill Series) in trough between escarpments, heather, sedges and Sphagnum moss, flat, boggy site with open pond and channels.

- **Site 3 01** (0 - 20cm) was fibrous wet peat in excess of 1 m thick. All black (5YR 2.5/1).

Site 4 Disused Magnesian Limestone quarry between Quarrington Hill and Old Quarrington (GR - 333379). OS Sheet 93, Teeside and Darlington. Brown rendzina (Elmton Series) on Permian Limestone, grassland vegetation, 4° slope with south-facing aspect.

- **Site 4 Ah** (0 - 15 cm) was a dark brown (7.5YR 3/3) humus rich loamy sand, which was very stony with abundant roots.
- **Site 4 R** (>15 cm) was light olive brown (2,5YR 5/4) weathered Magnesian Limestone with loamy sand matrix and abundant stones.

Garden soil was donated as a benchmark for validation experiments for comparison with the collected soils.

4.2 Characterisation techniques

The soils were individually characterised for organic matter, organic carbon, moisture, water holding capacity, carbonate, soil reaction/acidity, presence of soluble humic acids and cation exchange capacity (CEC). These properties have been shown to have substantial effects on the fate of organic compounds in soil [2-6]. The charaterisation techniques described below were taken from standard techniques used by the Soil Science Department at the University of Northumbria at Newcastle, unless otherwise stated.

4.2.1 Organic matter content

This was found by weight loss of soil on ignition at 800 ^{o}C for 45 mins. The mass lost on ignition is related to the organic content of certain soils, such as sandy soils that contain little or no clay, or chalky material, peats and organic clays containing more than about 10 % organic matter. However, it should be recognised that, in some soils, factors

unrelated to organic content could be responsible for the major portion of mass lost on ignition.

Procedure: Weigh 5 g soil into small crucible (W_1) and place in pre-heated muffle furnace (800 °C) for 45 mins. Remove the crucible and cool in a desiccator. Re-weigh sample (W_2) and calculate organic matter as follows:

$$\% \text{ organic matter} = \frac{(W_1 - W_2)}{W_1} \times 100 - \% \text{ moisture} \qquad \text{(eqn. 4.1)}$$

4.2.2 Organic carbon content

This was found using a modified Kurmies method as described in reference [7]. Kurmies method involves treatment of the soil with concentrated H_2SO_4 to remove inorganic carbon (as carbon dioxide) and wet oxidation of the soil organic matter with potassium dichromate ($K_2Cr_2O_7$) followed by spectrophotometric measurement of the green Cr^{3+} complex which is formed in proportion to the % organic carbon on the soil.

Reagents: Potassium dichromate solution (0.333 mol/L) was prepared by dissolving 98.06 g of $K_2Cr_2O_7$ in a mixture of 300 ml water and 100 ml concentrated H_2SO_4, and then diluted with distilled water to 1-litre in a volumetric flask. A substance of exact known carbon content 'sodium oxalate ($Na_2C_2O_4$)' was used as a standard in the oxidations.

Oxidation procedure: Weigh an amount of soil, containing organic matter in the range of 80 - 150 mg, and transfer it to a dry 250 ml volumetric flask. Weigh 1005 mg of sodium oxalate and transfer it to a dry 250 ml volumetric flask. Include also 2 blanks. Add to each flask 25 ml 0.333 M $K_2Cr_2O_7$ solution. Swirl a few times during the next five minutes then put the flasks in a sink and carefully add 40 ml concentrated H_2SO_4. Place the flasks in a boiling water bath for 1.5 hours swirling gently every 15 minutes. After this time cool each flask in the sink and make up to the volume with distilled water.

Preparation of soil samples for measurement: Pour a portion of the soil suspension into a plastic centrifuge tube and centrifuge for 10 minutes at 3000 rpm. Decant the clear supernatant into glass test tubes.

Preparation of standard series: Pipette from the 250 ml flask containing sodium oxalate standard, respective volumes of 0, 25, 50, 75, and 100 ml solution into 100 ml volumetric flasks. Make up to the mark with the blank and mix. Pour portions of the respective standard solutions into glass test tubes. This standard series corresponds to 0, 5, 10, 15 and 20 mmol Cr^{3+} per litre of solution.

Specrophotometric measurements: Measure the absorbance of the standard solutions and the sample supernatants in a 4 ml cuvette at a wavelength of 590 nm within 24 hours after oxidation. Plot the absorbance values for the standard solutions against the calculated Cr^{3+} concentrations, and read the Cr^{3+} concentrations for the soil sample supernatants. Calculate the percentage organic carbon in the soil samples by multiplying the Cr^{3+} concentrations found by 0.2250/**w**, where '**w**' is the weight of the oven dry soil sample.

4.2.3 Moisture content

Samples are weighed, oven dried and re-weighed. % moisture is established by weight loss.

Procedure: Weigh 30-50 g soil into a beaker, note weight of soil (W) and weight of soil + beaker (W_1). Dry sample in oven at 105 °C for 24 hrs. Allow sample to cool in desiccator, and re-weigh the beaker (W_2):

$$\% \text{ soil moisture} = \frac{(W_1 - W_2)}{W} \times 100 \qquad \text{(eqn. 4.2)}$$

4.2.4 Water holding capacity

This is an approximate measure of the potential that a soil has towards water saturation.

Procedure: c.a. 1 g soil is weighed into a glass test tube (W_1). The tube is weighed after soil addition (W_2). 3 ml (3 g) of water are added to the soil and the tube is then sealed and shaken for 2 days. After this time the tube is centrifuged at 2000-3000 rpm for 20 mins and the excess supernatant water is decanted. The tube is then re-weighed (W_3) and the % of water held by the soil is calculated by the weight difference and normalised to the weight of the soil.

$$\% \text{ 3 ml water held by 1 g soil} = \frac{1}{W_1} \times \frac{(W_3 - W_2)}{3} \times 100 \qquad \text{(eqn. 4.3)}$$

4.2.5 Carbonate content

Procedure: Weigh 5-10 g soil into plastic weighing boat (W_1). Pour 20 ml 10 % HCl into a small beaker, weight this and add to it the weight of soil (W_2). Tip the soil into the acid, swirl to mix and leave until effervescence has died down. Re-weigh beaker (W_3) :

$$CaCO_3, \text{ carbonate content } \% = (W_2 - W_3) \times (227/W_1) \qquad \text{(eqn. 4.4)}$$

4.2.6 Soil acidity

pH is often determined in a soil suspension made with distilled water, although it is suggested that a more realistic measure is obtained from a suspension made with 0.05 M $CaCl_2$ as this has a 'balancing' effect on the soil exchange complex.

Procedure: Weigh 10 g soil into a small beaker and add 25 ml distilled water (and repeat using 25 ml 0.05 M $CaCl_2$), stir and allow samples to stand for 5 mins. Calibrate pH meter with pH 4 and pH 7 buffers before immersing the pH electrode into the suspension. Allow reading to stabilise and record.

4.2.7 Cation exchange capacity (CEC)

This is a measure the amount of negative charge on the soil exchange complex and therefore a measure of a soils capacity to retain and supply cationic nutrients to plants and is often used as a measurement of soil fertility.

(1) Cations sorbed on the soil exchange complex must be displaced into solution and replaced by single cation species, preferably one which is not usually present in soils. This is usually achieved by treatment of the soil with a $BaCl_2$ solution, which is buffered at pH 8.1. Following this treatment the soil exchange complex is dominated by Ba^{2+} ions.

(2) The sorbed Ba^{2+} ions are then similarly displaced by treatment of the soil with $MgSO_4$ solution, after which the exchange complex is dominated by Mg^{2+} ions.

(3) The CEC is then derived from the amount of Mg^{2+} lost from the treatment solution used in (2) above. The Mg^{2+} lost from the treatment solution should be equivalent to that sorbed by the soil exchange complex.

Equipment and reagents;

Centrifuge bottles (200 ml),

Centrifuge,

Shaker,

Pipette (50 ml and 5 ml),

Burette, stand, clamps, white ceramic tile,

Small volumetric flasks (100 ml),

Distilled water,

Buffered $BaCl_2$ solution - mix equal volumes of the two solutions:

(a) Triethanolamine solution - 90 ml diluted to 1 litre and pH adjusted to 8.1 by adding approximately 140 ml HCl (2M). Dilute to 2 litres, mix and protect from CO_2 during storage,

(b) $BaCl_2$ (244 g/l $BaCl_2.2H_2O$),

$MgSO_4$ (6.2 g/l $MgSO_4.7H_2O$),

EDTA solution (3.723 g/l EDTA - disodium salt),

NH_4OH (140 ml/l 30 % w/w NH_3),

Indicator - Omega chrome black 0.1 % (freshly prepared).

Procedure: Weigh exactly 5 g of soil into a centrifuge bottle and note mass of soil + bottle (M_1). Treat calcareous and saline soils with 100 ml buffered $BaCl_2$ reagent and shake for 1 hour. Centrifuge at 3000 rpm for 10 mins and discard the supernatant liquid. NB - for non-calcareous and non-saline soils this first treatment may be omitted. Treat soil with a further 200 ml buffered reagent, shake overnight and discard the supernatant liquid. Add 200 ml distilled water and shake to break up the soil cake. Centrifuge at 3000 rpm for 10 mins and discard the supernatant liquid into a storage container/bottle. Refrigerate until ready for analysis (titration). Pipette a 5 ml sample of the above liquid into a small volumetric flask. Add 6 drops of NH_4OH and 2 drops of indicator to give a pinkish colour. Titrate with EDTA - colour changes from pink via clear to purple at end point (note volume in ml - B). The soil titration (A_1) must be corrected for the effect of the volume of liquid retained by the centrifuged soil after the water wash:

$$\text{Corrected titration in ml } (A_2) = A_1 (100 + M_2 - M_1) / 100 \qquad \text{(eqn. 4.5)}$$

CEC of the soil is then calculated as follows:

$$CEC = 8 (B - A_2) \qquad \text{(eqn. 4.6)}$$

The units are milliequivalents per 100 g (me/100 g) or centimoles of positive charge (from Mg^{2+}) per kg of soil ($cmol_{(+)}\ kg^{-1}$); (NB-$cmol_{(+)}\ kg^{-1}$ = me/100 g). The amount of Mg^{2+} calculated to be on the soil exchange complex therefore directly relates to its 'net' negative charge.

4.2.8 Presence of soluble humic acids

Humic acids in solution precipitate below pH 2, whilst fulvic acids are much more soluble and do not. Due to their nature, humic acids are generally extracted from soil under alkaline conditions. However, the procedure used here to extract humic acids mirrors the conditions used to extract phenols (chapter 5.0) since the presence of humic acids is important for the interpretation of the chromatography in chapter 5.0.

Procedure: c.a. 1 g of soil is weighed into a 100ml glass screw top flask. 50 ml of 60:40 MeOH:H_2O is added to the soil and the flask is shaken for 30 mins. The extract is then filtered by Buchner filtration and acidified below pH 2 with concentrated H_2SO_4.

4.3 Results

Table 4.1 shows the results of the above characterisation techniques except for the presence of humic acids which were not quantified. The results for the presence of humic acids showed that on acidifying the extracts from all the soils, only site 1 H/Ah and site 3 01 (high organic matter soils) extracts formed a red/brown precipitate. Therefore, these soils can be considered to posses soluble humic acids.

Table 4.1

Soil characterisation results

		Average % moisture n = 2 (individual values)	Average % water holding capacity n = 4 (% RSD)	Average % organic matter n = 3 (% RSD)	Average % organic carbon n = 3 (% RSD)
Site1	**H/Ah**	**8.33** (7.96, 8.70)	**60.69,** 9.46	**48.31,** 4.18	**35.75,** 4.16
	Ea	**0.14** (0.14, 0.14)	**17.27,** 2.44	**2.32,** 4.09	**1.31,** 5.92
	Bhs	**1.36** (1.35, 1.37)	**17.98,** 8.62	**4.26,** 9.93	**1.64,** 5.25
	C	**0.65** (0.64, 0.66)	**19.61,** 4.12	**2.24,** 6.34	**1.17,** 5.30
Site 2	**Ah**	**1.96** (1.94, 1.98)	**28.31,** 3.20	**13.60,** 2.39	**6.58,** 3.30
	Bg	**1.14** (1.14, 1.15)	**19.02,** 10.26	**7.36,** 0.53	**2.73,** 4.67
	BCg	**0.88** (0.87, 0.90)	**21.26,** 2.49	**4.50,** 1.71	**0.92,** 6.77
Site 3	**1**	**19.40** (18.05, 20.76)	N.D.	**75.32,** 3.54	**45.43,** 2.98
Site 4	**Ah**	**1.48** (1.46, 1.51)	**20.29,** 1.54	**17.52,** 0.89	**1.55,** 7.20
	R	**0.11** (0.09, 0.11)	**15.35,** 8.15	**17.18,** 7.67	**0.23,** 11.42
Garden soil		**1.86** (1.80, 1.92)	**23.34,** 2.26	**10.44,** 3.26	**3.77,** 1.45

Table 4.1 (continued)

Soil characterisation results

		Average % Carbonate n = 4 (% RSD)	Average pH measured in H_2O n = 2 (individual values)	Average pH measured in 0.05M $CaCl_2$ n = 2 (individual values)	Average CEC me/100g n = 2 (individual values)
Site1	**H/Ah**	**4.54,** 7.97	**3.94** (3.90, 3.98)	**2.97** (2.89, 3.05)	**89.82** (90.85, 88.80)
	Ea	**5.22,** 7.43	**4.41** (4.37, 4.46)	**3.51** (3.45, 3.57)	**1.53** (0.56, 2.50)
	Bhs	**5.77,** 8.82	**4.37** (4.31, 4.44)	**3.89** (3.81, 3.95)	**13.22** (10.77, 15.67)
	C	**3.11,** 7.13	**4.53** (4.49, 4.57)	**3.98** (3.94, 4.02)	**14.65** (14.66, 14.64)
Site 2	**Ah**	**7.43,** 3.79	**5.70** (5.65, 5.76)	**4.61** (4.53, 4.67)	**24.80** (24.39, 25.20)
	Bg	**0.59,** 8.61	**5.54** (5.53, 5.55)	**4.33** (4.28, 4.36)	**14.32** (12.63, 16.00)
	BCg	**0.54,** 7.47	**5.10** (5.15, 5.04)	**4.14** (4.09, 4.19)	**10.57** (6.23, 14.92)
Site 3	**1**	N.D.	**3.59** (3.47, 3.72)	**2.91** (2.86, 2.96)	**113.20** (125.07, 101.23)
Site 4	**Ah**	**22.58,** 0.77	**7.98** (7.85, 8.11)	**7.25** (7.20, 7.30)	**13.90** (11.65, 16.14)
	R	**21.43,** 1.69	**8.57** (8.51, 8.63)	**7.61** (7.57, 7.65)	**3.36** (4.00, 2.71)
Garden soil		**5.99,** 1.67	**6.61** (6.53, 6.68)	**6.09** (6.04, 6.14)	**24.79** (25.34, 24.24)

4.4 Discussion

The characterisation results in table 4.1 show that the soils collected have widely contrasting properties. For example site 4 soils were sandy and as expected have low % organic carbon and CEC, high % carbonate and alkalinity. In comparison, soil from site 3 (peaty degrading vegetation) had high % organic carbon, high CEC and acidity and no carbonate. Lower pH's were measured when soil was in an aqueous solution of 0.05 M $CaCl_2$. This may be attributed to proton release from the matrix via calcium ion substitution. In soils with high organic matter contents, the majority of the acidity measured will be due to functional groups in humic and fulvic acids with low pKa's [8].

These soils will be used for further sorption experiments and their characteristics related to the ultimate fate of the organic pollutants studied in varying locations.

4.5 References

1. W. Karcher, G. Kuhnt, M. Herrmann and H. Muntau, Proceedings of the 5th International workshop Environmental behaviour of Pesticides and Regulatory Aspects, A. Copin, G. Houins, L. Pussemier, J.F. Salembier, Eds.; Brussels (1994) p112, April 26-29.
2. R. Calvet, M. Leistra, In '*Interactions between herbicides and the soil*'; R.J. Hance, Ed.; Academic Press, London. **Chap. 1 & 2** (1980).
3. S.M. Lambert, *Weeds,* **14** (1966) 273.

4. D.B. Fenn and M.M. Mortland, *Proc. Int. Clay Conf.*, Madrid, (1972) 591.

5. M.G. Stapleton, D.L. Sparks, and S.K. Dentel, *Environ. Sci. Technol.*, **28** (1994) 2330.

6. C.T. Chiou, L.J. Peters and V.H. Freed, *Science,* **206** (1979) 831.

7. I. Walinga, M. Kithome, I. Novozamsky, V.J.G. Houba and J.J. van der Lee, *Commun. Soil Sci. Plant Anal.*, **23** (1992) 1935.

8. Z. Wang, B.C. Pant and C.H. Lanford, *Anal. Chim. Acta*, **232** (1990) 43.

Chapter 5.0

Shake flask extraction of phenols from aged soils

5.1 Experimental

5.1.1 Method validation using garden soil

A method was developed for the separation and analysis of phenol, 3-methylphenol, 4-ethylphenol and 1-naphthol by isocratic reversed phase HPLC with UV detection. The optimised method parameters for separation of the phenols were given in chapter 3. Acetic acid (1 %) was added to the mobile phase to ensure that the phenols were maintained as neutral compounds.

Initial method validation studies focussed on the potential sorption of the phenols to glassware and the reproducibility of the HPLC system. As part of the reproducibility study, standards were stored in glass volumetric flasks and analysed over a 40 day period. However, for calibration purposes, a new series of standards were freshly prepared on each day of analysis.

The sorbent in initial experiments was air dried garden soil. Three different masses of soil (c.a. 33 g, c.a. 61 g and c.a. 122 g) were employed to test the homogeneity of the spiking procedure. Soils were slurry spiked with the phenols chosen to give a typical mass of 1 mg phenol/g soil. Slurry spiking was accomplished by immersing the soil in DCM-phenol solution and magnetically stirring the soil until the DCM had evaporated.

Slurry spiking with DCM affords rapid removal of the solvent due to its low boiling point and deposition of the phenols onto dry soil surfaces. The finite time before total evaporation of the DCM will no doubt govern the initial extent of diffusion into the soil matrix. Due to lack of soil hydration during storage, diffusion into the pore structure may be attributed to vapour phase sorption/desorption interactions.

As DCM is not miscible with water it is assumed that the moisture content of the air dried soils remained constant after evaporation of this solvent (although this was not confirmed). The slurry spiked garden soil in the initial experiments was aged in ambient light and temperature in sealed glass jars in the laboratory.

An automated shake flask extraction method was used based on the British Gas Method for determining phenols in soils [1], where no heat or pressure contribute to the extraction. The method involved extracting 1 g of soil with 50 ml solvent (60 % MeOH : 40 % H_2O) in 100 ml screw top bottles. The use of MeOH as an organic co-solvent for extraction has been reported to be beneficial to analyte desorption both thermodynamically by enhancing solubility [2-3] and kinetically by softening and swelling the humin-kerogen polymeric natural organic matter structure [4]. The bottles were shaken for 30 min on a Warburg mixer (rotating disk). After this time the solution was filtered by Buchner filtration (suction). The bottle and Buchner flask were rinsed with solvent which was added to the extractant before making up to a final volume of 100 ml. An aliquot of this solution was then filtered through a 0.45 μm membrane Acrodisk to remove finer sediment prior to HPLC

analysis. At each time point, extraction was repeated at least 5 times with each soil mass to account for the heterogeneity of the soil sample and the error involved in sample preparation.

The spiked soil was extracted at time periods which ranged from initial application to 187 days post application to monitor the effects of soil ageing and interaction time of the phenols. Spot spiking was also carried out on the soil in order to evaluate the analytical methodology i.e. to observe whether a maximum recovery could be extracted at the shortest possible time after application with the extraction and analysis method. Celite was chosen for comparison with soil as it has been used as an inert chromatographic support matrix since the inception of HPLC [5]. As a result, a full relative recovery was expected from Celite compared with soil. The procedure of 'spot spiking' was carried out on Celite and soil. Spot spiking involves adding the analyte in a small volume of solvent to a small mass of the matrix and allowing the solvent to evaporate prior to extraction of the analyte from the matrix. With this technique sorption behaviour can be observed after a short contact period between analyte and matrix. The Celite and soil were spot spiked (c.a. 1 g) with the same concentration of phenols spiked onto the bulk soil in a small volume of DCM (1 ml). Due to the volatility of the DCM and the smaller sample mass used, total evaporation occurred in c.a. 30 mins and the samples were extracted immediately.

5.1.2 Method application to collected soils

After the above initial validation studies with garden soil, the slurry spiking method was repeated to include phenol, 4-methylphenol, 4-ethylphenol, 2-naphthol and 1-naphthol.

The matrices used in further studies included garden soil and the soils collected and characterised (as detailed in chapter 4.0). Due to the complexity of the soils studied, sand and Celite were also included in these sorption experiments, as simple matrices. A standard mass of c.a. 70 g of all the matrices was slurry spiked with the phenols in DCM. The matrices were aged in the dark in sealed glass jars at ambient temperature and extracted over time periods that extended from initial application to c.a. 250 days post application. The extraction method parameters remained the same and each matrix was extracted 4 times at each time point.

Capacity factors were calculated for the phenols, possible degredation products and possible humic/fulvic acids. The capacity factor (K or K_{HPLC}) is a measure of the time an analyte spends in the mobile phase and the stationary phase of a chromatographic column. The capacity factor can be calculated from the retention times in mins of the peak of interest (t_R) and an 'unretained' peak, i.e. solvent (t_0), by the equation below:

$$K = \frac{t_R - t_0}{t_0} \qquad \text{(eqn. 5.1)}$$

5.1.3 Radiolabelled spiking

To determine whether degredation products may form which may be extracted yet not detected by UV absorbance at the set wavelength, the radiolabelled analogue of one of the phenols (1-naphthol) was chosen to be spiked onto garden soil and Celite prior to extraction over time. The detection method of liquid scintillation spectrometry (LSS) was used which

measures radioactivity independent of compound type. Radiolabelled 1-naphthol was spiked onto the garden soil and sand (c.a. 2 μg/g) which were then aged in sealed jars in the dark. Extractions were carried out by shake flask at 4 and 130 days post application using the same extraction procedure described previously. The garden soil and sand were extracted in triplicate at each time point. Blank garden soil and sand were also extracted and analysed to determine background radiation. A control containing the concentration of 1-naphthol which would lead to 100 % response and hence recovery was also included. Each extract was filtered by suction and an aliquot fitered through a 0.45 μm membrane Acrodisk filter. 1 ml of this filtrate was added to 1.5 ml of liquid scintillation cocktail in a 5 ml plastic screw top vial. Each vial was shaken to mix the contents and placed in the autosampler of the LSS and analysed. The method of radiolabelled compound analysis by LSS is described in greater detail in the next chapter.

5.2 Results

5.2.1 Method validation using garden soil

The analysis of standard phenol solutions over 40 days showed that negligible losses could be attributed to sorption to glassware over this period. RSD's ranged from 2.0 % for phenol to 3.7 % for 1-naphthol for the 10 ppm standard whilst for a 1 ppm standard RSD's ranged from 3.6 % for phenol to 5.8 % for 1-naphthol. The chromatograms showed no signs of degradation products emerging during this time period at 275 nm. Spot spiking of Celite and soil, followed by extraction gave average % recoveries (n = 5) shown in table 5.1.

Table 5.1

Recoveries (%) from spot spiking Celite and garden soil

	Celite	Garden soil
Compound	% Recovery	% Recovery
phenol	84.9	87.5
3-methylphenol	99.6	95.7
4-ethylphenol	101.5	88.5
1-naphthol	102.8	77.4

It can be seen that the recovery of the phenols from Celite is acceptable with a slightly low recovery for phenol. However, when the same spot spiking procedure followed by extraction was applied to garden soil, an immediate lower recovery (77.4 %) of 1-naphthol is observed.

The results for % recovery of the phenols extracted from the three masses of garden soil were independent of soil mass. The results for % extraction recovery of phenols from garden soil (c.a. 122 g) are shown in table 5.2.

Table 5.2

Extraction recoveries of phenols from garden soil v's time since application.

	Average % extraction recovery (n = 4)			
Time/days	**Phenol**	**3-methylphenol**	**4-ethylphenol**	**1-naphthol**
0 (Celite)	84.9	99.6	101.5	102.8
0 (garden)	87.5	95.7	88.5	77.4
1	82.6	85.7	88.4	27.6
7	84.7	88.7	91.2	15.3
21	83.4	87.4	89.0	5.9
28	84.4	91.8	91.7	5.1
187	58.3	86.8	81.9	2.2

During the first few days post application there was a high initial loss of all the phenols on the soil. This was followed by no further significant loss of 3-methyl and 4-ethylphenol over the 187 days. Phenol was slowly lost with time and 1-naphthol was rapidly lost.

RSD's (n=4) for the ageing and extraction of phenols from garden soil over the time period of 187 days ranged from 1.6 to 7.3 % for phenol and 2.0 to 26.7 % for 1-naphthol. The low concentrations of 1-naphthol extracted posed limitations on detection leading to a wider range of RSD's. Rapid sorption will also exhibit greater uncontrollable fluctuations correlated with soil heterogeneity leading to higher RSD's. There were no obvious correlations between RSD's and mass of soil spiked or time of extraction suggesting that 1 g of the bulk matrix is homogeneous for masses between 33-122 g.

5.2.2 Method application to collected soils

Table 5.3 below shows the range of RSD's for the experimental procedure for all the soil analysed over the time period of 250 days.

Table 5.3

The RSD's calculated for the repeats of each soil extraction

	Range of % RSD's for extractions (n=4)				
Site	**Phenol**	**4-methylphenol**	**4-ethylphenol**	**2-naphthol**	**1-naphthol**
Garden	1.6 - 4.0	2.7 - 4.1	1.7 - 3.8	1.6 - 5.4	2.4 - 6.1
Celite	1.0 - 3.8	0.5 - 3.6	0.9 - 4.5	1.8 - 8.1	11.4
Sand	2.6 - 9.1	2.0 - 9.1	2.1 - 9.4	4.3 - 9.0	2.9 - 8.4
1 H/Ah	2.3 - 5.7	3.0 - 5.4	1.4 - 5.6	0.6 - 4.7	2.3 - 8.2
1 Ea	3.6 - 7.2	4.1 - 7.6	3.9 - 6.9	3.4 - 5.5	3.9 - 5.1
1 BhS	13.4 - 16.6	12.9 - 15.2	12.8 -14.8	13.4 - 15.9	14.1 - 17.1
1 C	3.2 - 5.3	2.8 - 4.8	2.7 - 5.6	1.7 - 5.4	1.0 - 8.9
2 Ah	1.6 - 4.4	1.9 - 4.0	2.1 - 4.9	1.6 - 7.0	2.3 - 5.1
2 Bg	2.5 - 8.2	2.8 - 7.8	2.5 - 6.3	1.8 - 4.8	0.6 - 6.3
2 BCg	1.5 - 4.9	1.1 - 4.7	2.6 - 4.0	2.1 - 4.8	3.4 - 4.9
3 01	1.4 - 7.1	1.2 - 7.3	1.7 - 8.2	0.8 - 8.1	0.7 - 8.3
4 Ah	2.6 - 9.8	2.3 - 9.3	2.4 - 10.1	2.3 - 6.7	0.9
4 R	2.1 - 5.1	2.0 - 5.6	1.5 - 4.5	2.2 - 4.3	5.0

The RSD's (table 5.3) show that the experimental procedure is repeatable. However, the high RSD's for site 1 Bhs suggest that soil heterogeneity may lead to inaccurate results. This heterogeneity was observed with the RSD's for soil characterisation, where 1 Bhs had high RSD's for % organic matter.

When presented graphically, the results of these experiments showed that the sorption of the phenols on the soils fell into 5 similar groups/trends. The five trends are summarised in

the discussion. An example from each sorption trend is given on the following pages (figures 5.1-5.5). Chromatograms are shown below each graph for extractions carried out at 1 day and c.a. 250 days after spiking to highlight the sorption of phenols and the emergence of degredation products. Appendix A shows further graphs and chromatograms for the soils not shown here. The 'key' below identifies the phenols in the results on the following pages.

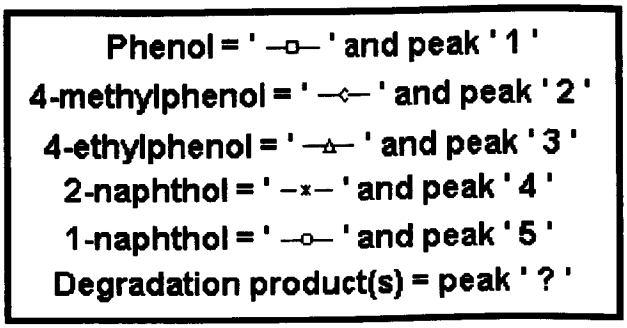
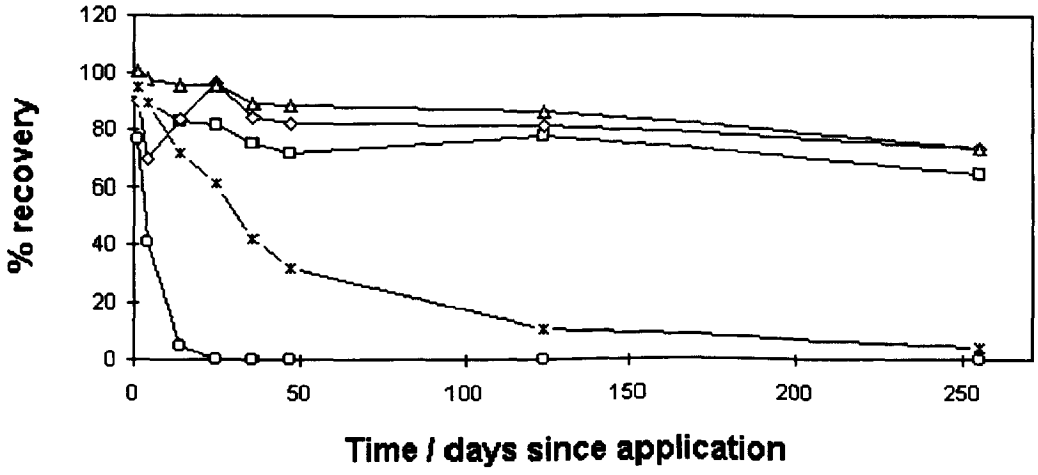


Figure 5.1

Influence of soil sorption time on the recoveries of phenols extracted from site 1 C (fine sand) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 1 C (fine sand) by shake flask.

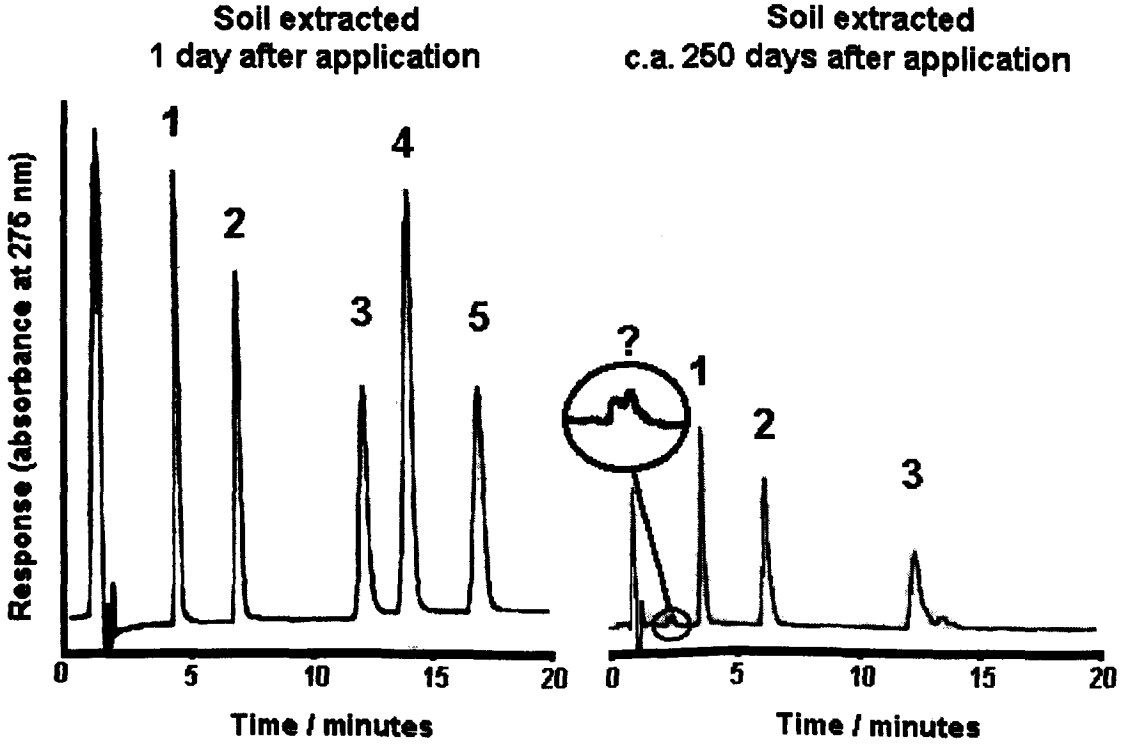
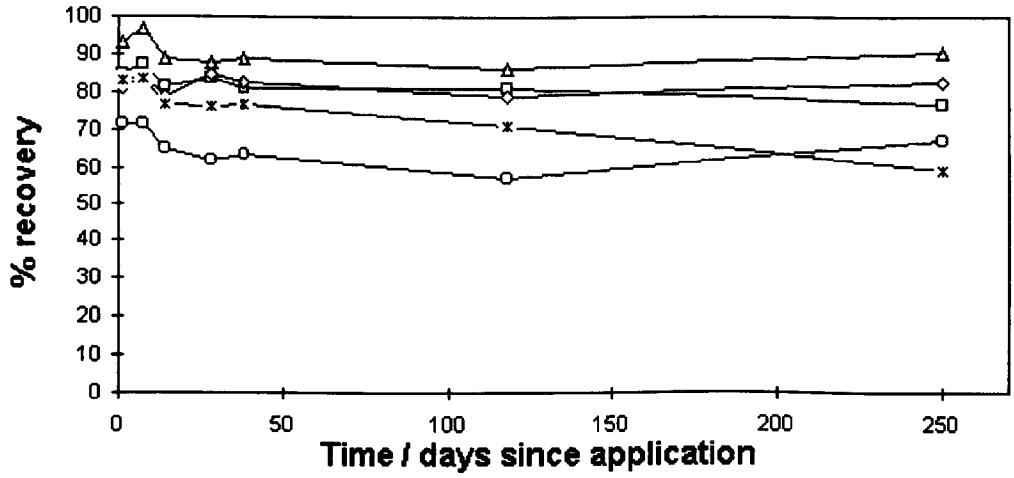


Figure 5.2

Influence of soil sorption time on the recoveries of phenols extracted from site 1 H/Ah (peaty/quartz grains) by shake flask



Influence of soil sorption time on the chromatography of phenols extracted from site 1 H/Ah (peaty/quartz grains) by shake flask.

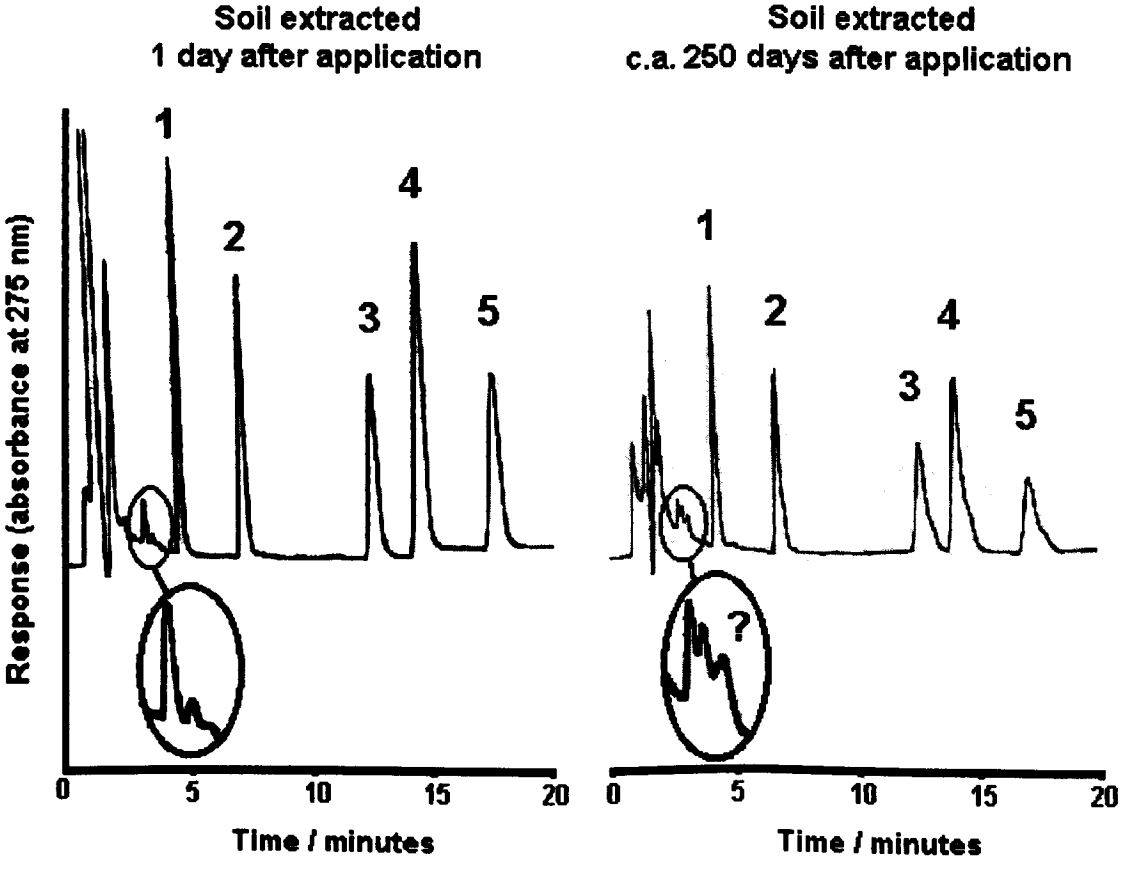
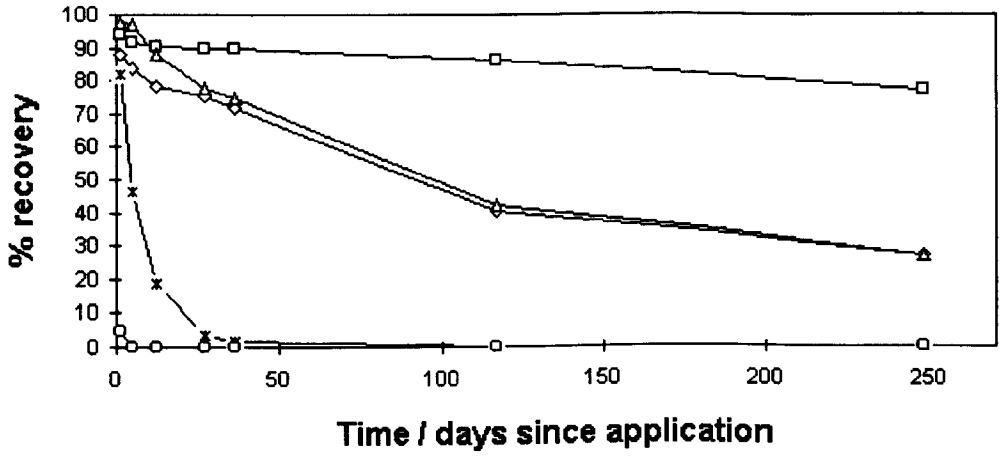


Figure 5.3

Influence of soil sorption time on the recoveries of phenols extracted from site 4 Ah (humus rich loamy sand) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 4 Ah (humus rich loamy sand) by shake flask.

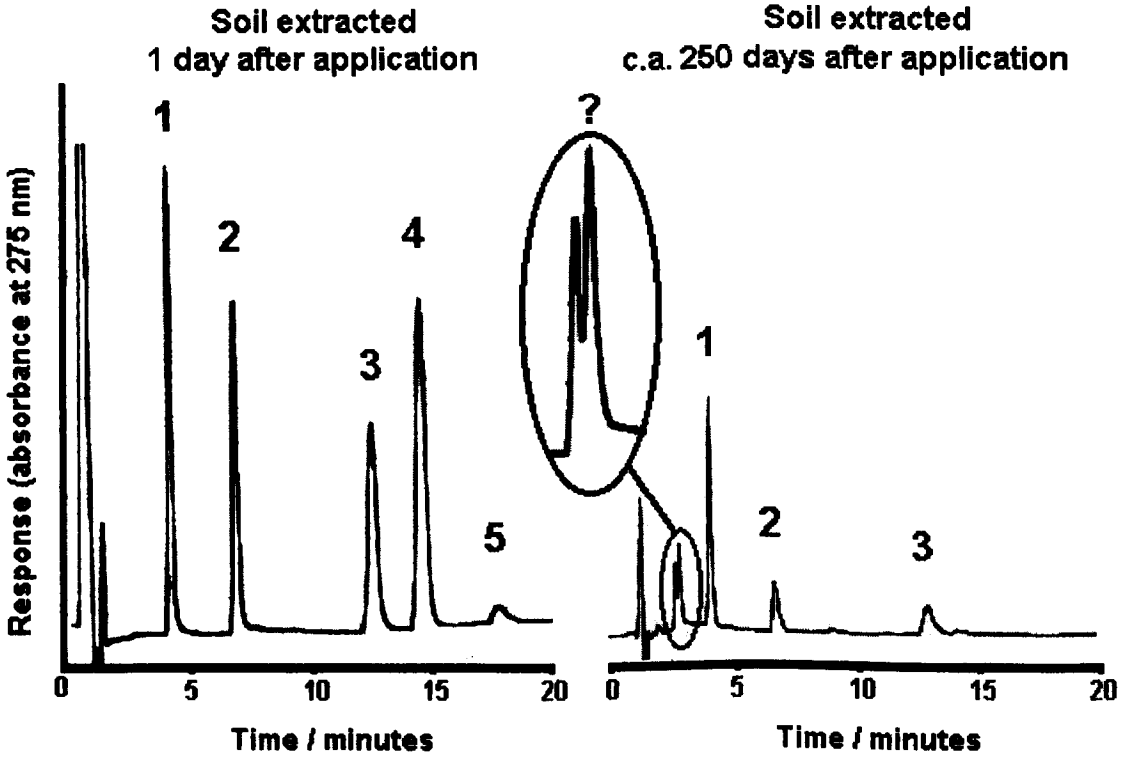
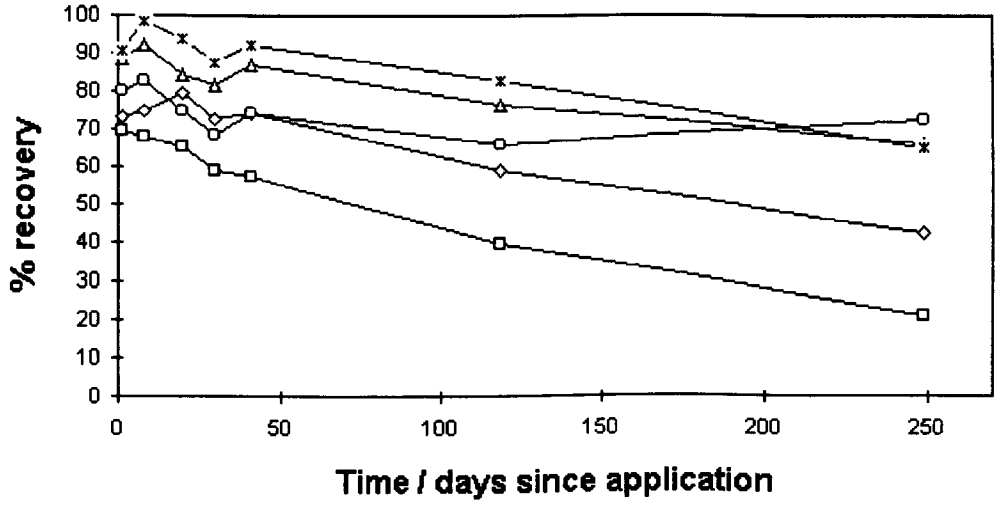


Figure 5.4

Influence of soil sorption time on the recoveries of phenols extracted from site 1 Ea (sandy/quartzy) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 1 Ea (sandy/quartzy) by shake flask.

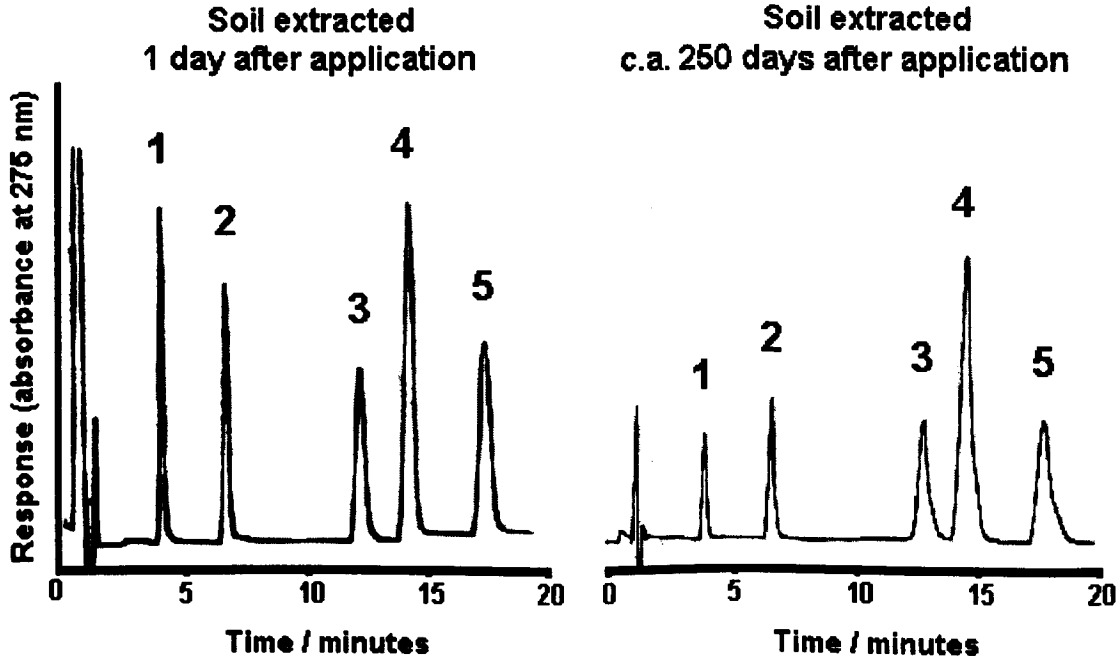
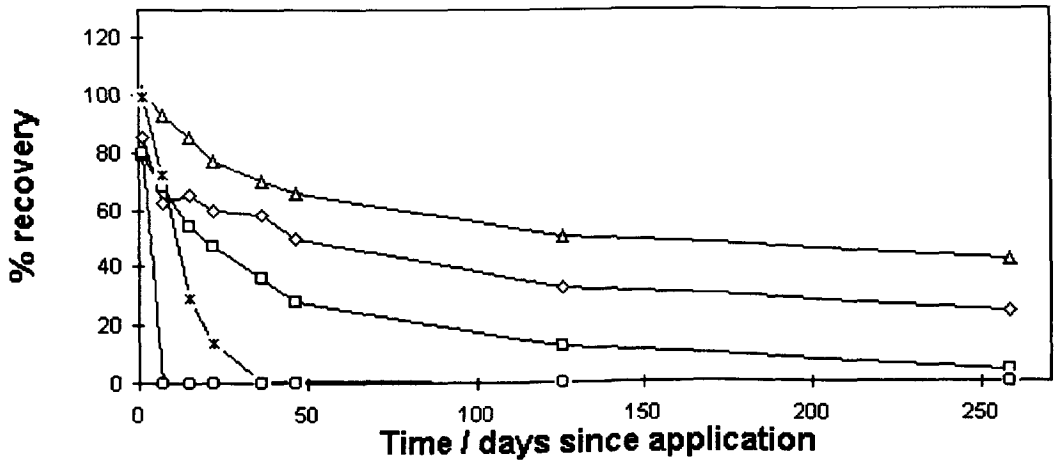


Figure 5.5

Influence of soil sorption time on the recoveries of phenols extracted from Celite by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from Celite by shake flask.

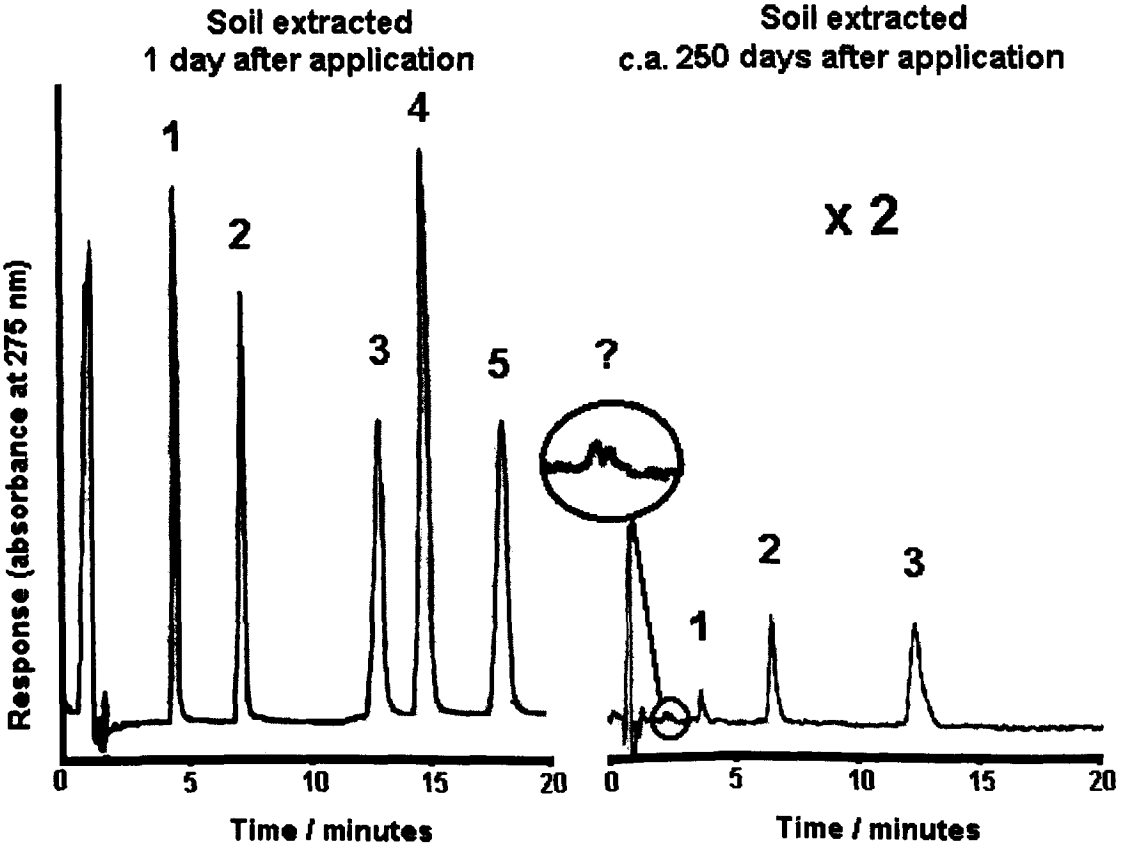


Table 5.4 below shows the capacity factors for the phenols measured in these experiments including the believed interferences due to humic acids and degradation products.

Table 5.4

Calculated capacity factors for the prominent chromatographic peaks

	Capacity factor
Humic peak 1	0.68
Humic peak 2	**0.76**
Degradation peak 1	**0.76**
Degradation peak 2	0.87
Phenol	1.48
4-methylphenol	2.88
4-ethylphenol	6.11
2-naphthol	6.95
1-naphthol	8.63

5.2.3 Radiolabelled spiking

Table 5.5 below shows the results for the extraction (and quantification by LSS) of radiolabelled 1-naphthol from spiked garden soil and sand at 4 days and 130 days post application.

Table 5.5

Recoveries (%) of 1-naphthol from garden soil and sand

	Average % recovery (n=3)	
Time/days	**Garden**	**Sand**
4	99.84	92.21
130	53.40	71.96

5.3 Discussion

5.3.1 Long term sorption results

- **Summary of the trends in sorption for each group**

(1) Site 1 C (Figure 5.1), 2 BCg, 2 Ah, 1 Bhs, Garden, and 2 Bg

1-naphthol >> 2-naphthol >> (phenol > ≅ 4-methylphenol > ≅ 4-ethylphenol)

After initial high sorption, phenol, 4-methylphenol, and 4-ethylphenol show no significant increase in sorption with ageing. There is a generally high sorption for 1-naphthol with slower sorption of 2-naphthol. This may be related to the lower pKa (acid dissociation constant) of the naphthols shown in table 5.6 below.

Table 5.6

The pKa's of the phenols studied

	pKa
Phenol	9.89 [6]
4-methylphenol	10.17 [6]
4-ethylphenol	10.2 [7]
2-naphthol	9.51 [8]
1-naphthol	9.34 [9]

All soils showed signs of degradation products emerging which were not present in soil blanks or standards.

(2) **Site 1 H/Ah (Figure 5.2)** and 3 01

(1-naphthol ≅ 2-naphthol) ≻(≅ phenol ≅ 4-methylphenol ≅ 4-ethylphenol)

After initial high sorption, all phenols show no significant increase in sorption with ageing. There are two sorbing groups according to aromaticity. Since the naphthols are sorbed more than the phenols, this suggests weak hydrophobic sorption through aromatic π - molecular orbitals which would be greater in a 2 ring molecule. Soils showed signs of degradation products emerging which were not present in soil blanks or standards.

(3) **Site 4 Ah (Figure 5.3)**

1-naphthol > 2-naphthol > (4-methylphenol ≅ 4-ethylphenol) > phenol

Very fast sorption of 1- and 2-naphthol. 4-methylphenol and 4-ethylphenol show a similar and significant increase in sorption with ageing. Phenol shows very slow sorption with ageing. Sorption increases with increased hydrophobicity. Soil showed signs of degradation products emerging which were not present in soil blanks or standards.

(4) **Site 1 Ea (Figure 5.4)**

Phenol >> 4-methylphenol > 1-naphthol > 4-ethylphenol > 2-naphthol

All phenols show steady sorption with ageing which, in part, seems to be controlled by polarity. Specific sorption or competitive sorption to specific sites or other factors may also contribute. Soil showed no sign of degradation products emerging over the time course of the experiments.

(5) **Celite (Figures 5.5)**, sand and site 4 R

1-naphthol > 2-naphthol > phenol > 4-methylphenol > 4-ethylphenol

Continued sorption with ageing. Sorption increases with increased acidity and becomes less with decreased acidity. It was observed that spiked Celite changed colour from white to a purple/brown on ageing. This colour change was very difficult to observe on the soils, which themselves are shades of brown. Celite, sand and site 4 R showed signs of degradation products emerging which were not present in blanks or standards.

Comparing the soil characteristics listed in table 4.1 with the sorption 'patterns' over time of each phenol, it can be observed that no single soil characteristic measured is governing sorption. Phenol appears to be sorbed to low moisture, low organic carbon and low CEC soils. Sorption of phenol doesn't appear to depend on the soil pH or % organic matter.

Likewise, the sorption of 4-methylphenol and 4-ethylphenol is not solely controlled by one soil characteristic, but seems to sorb more to low organic carbon, low moisture, high carbonate, low acidity soils. The sorption of 2-naphthol and 1-naphthol is difficult to interpret because they appear to prefer to sorb on low moisture, low organic carbon soils however they are also least sorbed by site 1 Ea (fig 5.4) which displays most of these characteristics. The acidity, CEC and carbonate content of the soils don't appear to be important in the sorption of naphthols.

Naphthol disappeared from the extracts for sites 4 R, 4 Ah , 1 C, 1 Bhs, 2 BCg, 2 Bg, Celite, sand and garden soil, at various times ranging from 5 days for site 4 Ah to 118 days

for 2 Bg. Sites 1 H/Ah, 3 01 showed small loss of naphthol and possible degradation products whilst site 1 Ea (containing large quartz crystals) showed minimum loss of naphthols and was the only soil which clearly had no sign of degradation products at 250 days after spiking. This also suggests that the degradation products are derived from 1-naphthol (and 2-naphthol) and not phenol. It was surprising to observe high sorption of phenols on sand and Celite followed by emergence of degradation products. As Celite is considered to be sterile this suggests that bacterial or fungal action is not required to form this product and may also highlight the sterility of the soils.

The degradation products began to emerge in site 4 Ah at 27 days compared to 120-250 days for most of the other soils. This coincides with the high sorption of 1-naphthol.

The degradation products can not be quantified at present due to their unknown identity. However, visible comparison of the peak heights of degradation products at c.a. 250 days show the following trend:

4 Ah >> Garden ≅ Sand ≅ 4 R > 2 Ah ≅ 2 BCg ≅ 2 Bg > 1C ≅ 1 BhS > Celite.

Two early eluting degradation products are detected in all the soils. However, these products are poorly resolved from early eluting matrix co-extractives (also observed in the blanks) from the high % organic matter soils 'site 1 H/Ah' (fig. 5.2) and 'site 3 01 (Appendix 1, fig A6)'. The chromatograms of low % organic matter soils are free from the early eluting matrix co-extractives found in the high % organic matter soils. Soil characterisation by acid

precipitation confirmed the presence of soluble humic acids in site 1 H/Ah and site 3 01 soils (chapter 4.0) which may therefore be the matrix co-extractives observed.

Celite was used for spot spiking and slurry spiking in these experiments based on the initial assumption that it was a matrix which would not allow the irreversible sorption of phenols (i.e. it was inert). However, phenols are irreversibly sorbed and degraded by Celite (and sand). It has been found that the sorption in clay systems of acidic compounds is dependent on solution pH whilst the sorption of basic compounds is dependent on the surface acidity of the sorbent [8]. Phenols are weakly acidic and due to the application method, sorption may depend on surface acidity of clays in the drier soils. The reason for this may be analogous with the fact that in the aqueous environment, water in direct contact with a negatively charged surface (such as SiO_2) will increase in acidity due to extensive H-bond formation [9]. By the same rational, phenols at the surface of a negatively charged mineral will interact via H-bonding. This will in turn increase the acididity of the phenol at the surface by favouring proton dissociation. In a dry environment the resulting anion can not be hydrated and the process will either be reversed or the anion will interact with positive charged surface sites and may become covalently bound. The observation that the more acidic phenols bind to a greater degree to Celite and sand further mirrors this possible explanation.

Table 5.6 on the following page shows some chemical properties of the phenols used. (n.f. = not found). The log K_{HPLC} are calculated from the capacity factors previously mentioned and is a measure of hydrophobicity.

Table 5.6

Some chemical properties of the phenols studied

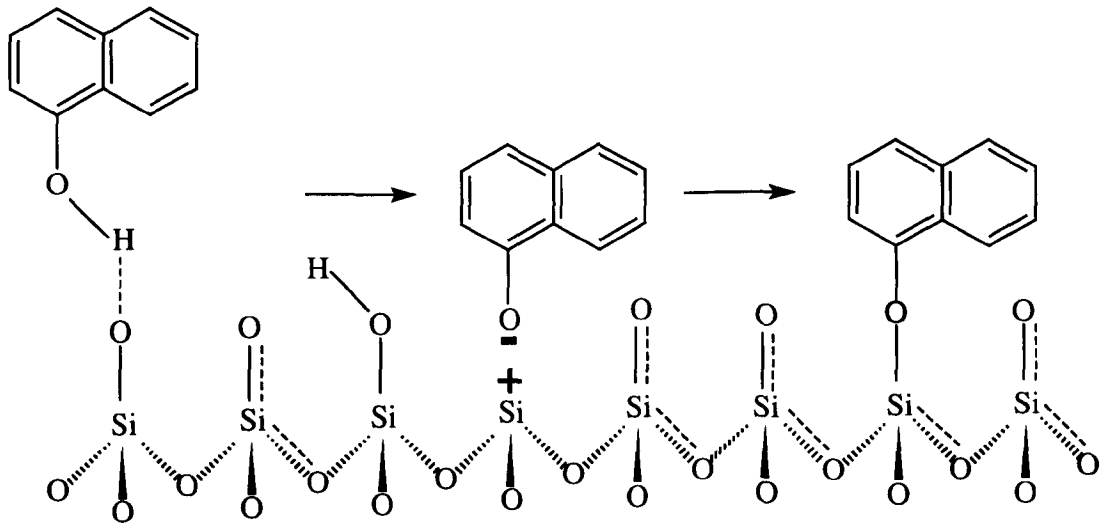
	pKa	Solubility g/L	log Kow	log K_{HPLC}
Phenol	9.89 [6]	93 [6]	1.46 [10]	0.17
4-methylphenol	10.17 [6]	23 [6]	n.f.	0.46
4-ethylphenol	10.2 [7]	n.f.	n.f.	0.79
2-naphthol	9.51 [8]	n.f.	n.f.	0.84
1-naphthol	9.34 [9]	0.866	2.9 [11]	0.94

Table 5.6 shows that 1-naphthol is more acidic compared with the other phenols and will therefore lose a proton more freely. As explained, this may lead to electrostatic coulombic interactions with positive sites on the negatively charged surface probably followed by covalent bond formation to enhance stability. This may be one mechanism which can favour the catalysis of the polymerisation of 1-naphthol as it would bring phenols into close proximity with each other. Covalent binding would also account for the lack of 1-naphthol and 2-naphthol being extracted from aged soils. Likewise, polymerisation would account for the eventual release of the 'polymeric degradation products' due to the extended repulsion from the negatively charged surface coupled with the high polarity of the compound (as mentioned with the lack of binding of humic acids or dissolved organic matter).

Therefore a possible pathway for 1-naphthol sorption (and other phenols) at the surface of Celite or sand can be visualised by figure 5.6 on the following page.

Figure 5.6

Possible 1-naphthol sorption on the surface of SiO_2



Hydrogen bonding followed by dissociation and covalent bonding may also be one important binding mechanism to the clay minerals of the soils studied. In quartz crystals the silicon is bonded tetrahedrally to oxygen atoms. The bulk material is inert as observed by the lower sorption of all phenols to the quartzy soil 1 Ea and the trend of higher acidity phenols sorbing to a higher degree doesn't dominate. However, the surface area of the SiO_2 increases from quartz through sand and to Celite and the material becomes more amorphous in Celite. In an amorphous structure such as Celite there are fewer geometrical constraints and as a result many Si atoms may be left with only 3 oxygen atoms and an unpaired electron profoundly disturbing the electronic structure of the material [12]. The possibility of surface silanol groups and free oxygens also make this an environment ideal for the interaction of phenols by H-bonding. As a higher rate of sorption occurs for all these phenols on Celite than sand, this too can be related to the structural quality of the material. Celite has a higher

surface area than sand but it is observed that sand produces a greater amount of the 'suspected polymeric material'. The reason for this could be due to;

- The relative ease of extraction of this material from sand compared with Celite due to diffusion controlled desorption of large molecules through the micropore structure of Celite being rate limiting

- More effective polymerisation on large crystal faces of sand (+ ease of release).

- Higher concentrations of catalytic impurities in sand able to propagate polymerisation.

- Limited polymerisation in the micro porous structure of Celite due to size restrictions.

Calcite may be one form of the $CaCO_3$ measured in the soils and possess a positively charged surface (chapter 1.0, table 1.2). One would expect calcite and other positively charged minerals to have a strong affinity for the sorption of phenols. It is not surprising that site 4 Ah which contains the highest amount of $CaCO_3$ (22.6 %) and also some organic matter (17.52 %) also has a large contribution to binding phenols strongly. 4 Ah binds in order of aromaticity and hydrophobicity (as with K_{HPLC}). The association between carbonate and organic bound layers has been made by Suess [13] and therefore initial binding may be due to partitioning into humic acids or dissolved organic matter bound to the $CaCO_3$. Under the high pH conditions of this site more acidic phenols will also show a degree of

dissociation to anions which will be favourable in binding to positive surfaces. Site 4 Ah also produced the highest amount of the degradation products and the later extracts were a rosy pink colour. Supporting evidence in the literature for a red/purple coloured polymer forming from 1-naphthol and 2-naphthol under various conditions has previously been discussed in chapter 1.0.

5.3.2 Radiolabelled spiking

At first sight, the results from the radiolabelled 1-naphthol experiment suggest that a high recovery of 1-naphthol is extracted from the garden soil and sand. However, such a high % recovery of 1-naphthol in the extract after 130 days can not be attributed to free 1-naphthol due to the high loss observed at this time on soil and sand by HPLC-UV. The reason for this is that the LSS technique measures radioactivity and is therefore not compound specific. Coupled with the results from the sorption experiments using HPLC-UV this suggests that 1-naphthol may be degraded or polymerised to a product which is extracted. This extracted product retains the radioactive ^{14}C of 1-naphthol but does not retain the UV absorbance or HPLC retention characteristics of 1-naphthol. Due to the filters used, the radioactivity in the extracts of garden soil can be attributed to free naphthol molecules or molecules bound to particles or soluble soil co-extractives larger than 0.45 μm. As the sand extract also shows a high amount of radioactivity this would suggest that the radioactivity is present as a degradation product. This is further evidence for the coincidence between 1-naphthol loss and the appearance of degradation products after 120 days in garden soil and sand in ageing experiments monitored by HPLC-UV.

5.4 Summary and conclusions

The results indicate that the sorption/desorption of the monocyclic phenols is a different process to that of 1-naphthol and 2-naphthol. The generally high extraction recoveries of the monocycles suggests physical sorption to sites on soil surfaces of different energies with a small resistant fraction perhaps retained by sites with deep potential energy wells resulting in high energy of desorption (Edes*) dictating the use of heat to facilitate release. Alternatively a resistant fraction may be diffused deep in the micropore structure causing diffusion limitations requiring increased solvation extraction times for release.

Other mechanisms for sorption could include surface sorption to non organic matter such as clay minerals. Comparing monocycles with 1-naphthol and 2-naphthol it can be seen that the most important factor governing sorption/desorption is increased aromaticity.

There must be a fundamental reason for the subtle differences in recovery between the monocycles and naphthols based around the extra ring. The extra ring affects the physical properties (size) and also contributes to the chemical properties of the molecule (table 5.6). The apparently irreversible sorption of the naphthols coupled with their possible degredation/polymerisation may be due to any one or several of the following mechanisms depending on the soil type;

- Trapping in inaccessible microsites within the soil matrix hindering molecular diffusion due to size restriction of naphthols and their degradation products.

- Site specific interactions or competitive sorption to soil sites with high sorption/desorption energies.

- Chemisorption to soil/mineral surfaces or clay inter layers, with possibility of partially catalysed breakdown or synthesis/polymerisation.

- Complexation (coulombic interactions) or incorporation (chemisorption) into soil organic matter with similar poly-aromatic groups such as humic/fulvic acids and lignins.

- Possible degradation pathways utilised by some bacterial populations in the soil unable to catabolise the monocycles to the same extent.

In the case of complexation, partitioning (physical sorption) or chemisorption into humic acids it is possible that shake flask may extract the complex from the soil matrix. The complex may pass through the 0.45 μm filter [14] and may have a low capacity factor and be eluted with the solvent on the chromatographic stationary phase due to its polar, acidic or polymeric nature. With the detection method of UV, 1-naphthol will remain undetected in a complex with humic acids due to retention and chromophore differences. The degradation products observed in the high organic matter soils could be due to the incorporation of 1-naphthol into soluble humic material with the result of a wavelength shift to 275 nm.

Celite and sand are both principally inorganic matrices of SiO_2, where Celite is consisted of fine porous particles with high surface area and sand is a primary mineral of

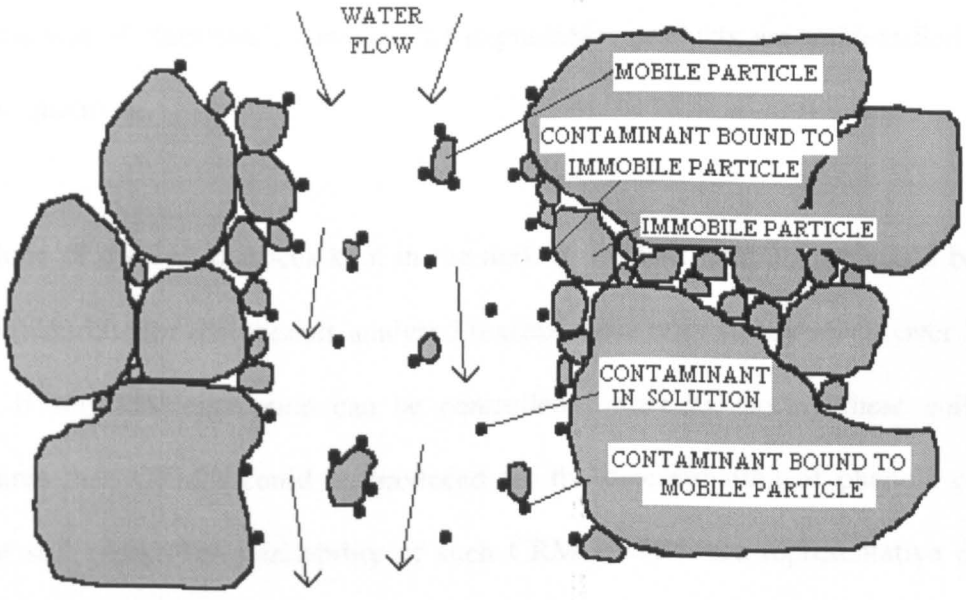
rock quartz with a well defined crystalline structure of much higher particle size. Although these matrices were not characterised they were not expected to have any humic matter present due to their negative surface charge previously mentioned (chapter 1.0, table 1.2). This suggests that for these matrices the emergence of degradation products is independent of the presence of humic or fulvic acids and may indicate the synthesis of naphtholic polymers with similar absorption and polarity as humic substances.

At first sight it seems that whichever process governs sorption of the naphthols, their toxic potential in soil diminishes with time, and leaching of the chemical into water supplies will be minimal. However, it is known that mobile colloidal particles can be released within soils and travel over large distances in the subsurface zone [15].

Recent studies suggest that organic compounds strongly bound to colloidal particles may be transported by these smaller particles under field conditions [16]. This is represented in the schematic diagram on the following page (figure 5.7), which shows contaminant transport within the soil pore structure.

Figure 5.7

Schematic diagram of colloid facilitated transport



Although 1-naphthol (and to a similar extent 2-naphthol) may be irrecoverable in most of the soils studied, their degradation products may be more toxic. They may also be bound more or less tightly to the soil than the naphthols depending on sorption mechanism or water solubility. Their fate under field conditions may be influenced by colloid-facilitated transport or partitioning/complexation with soluble humic acids. This will enhance movement of degradation products into the ground water and in the worst case scenario these mobile particles will ultimately transport tightly bound organic compounds into regions which may favour their release. This will ultimately place them in direct contact with aquatic life. It is shown in the next chapter that adverse wetting of garden soil can decrease particle sizes leading to an increase of very fine and possibly mobile plate-like particles. The lower degree of mediation of the monocyclic phenolics by most of the soils is of greater concern.

The high surface area and open porous structure of Celite has made it an ideal matrix for immobilising lipase enzymes for the modification of food fats [17] and phenol degrading biofilms [18]. Our studies show that dry Celite is an ideal structure for phenol degradation without the use of 'biofilms', however the degradation products are unidentified and may have toxic qualities.

None of the soil matrices kept in the dark at ambient temperature could be used as reference materials for the phenols analysed (except those with steady levels over long time periods). If sorption/degradation can be controlled further by storing these soils at low temperatures then CRM's could be produced for the determination of phenols on a wide variety of soil types. The availability of such CRM's which are representative of a wide selection of environmental soil horizons would be of great value to environmental analysts for the reasons discussed in the first chapter.

The absence of any observable clear distinction between soil properties measured and the sorption of the phenols makes it complicated to explain the mechanisms governing the sorption of phenols on the soils studied. Further work should be undertaken to characterise the soils for other properties including % clay mineral type (e.g. kaolinite and montmorillonite), metal oxides, quartz, ferrihydrite, and calcite, anion exchange, bulk density, and surface area (relating to pore structure). The aromaticity of humic materials present may also be valuable information. It may also be beneficial to apply the individual phenols to the soils in order to account for competitive binding.

The presence of degradation products which may be undetected at 275 nm in the soils is also cause for concern. The degradation products observed in the chromatography possess similar K_{HPLC} and absorbance intensity as the possible humic substances detected in high organic matter soils. This may be because they have similar polarity, size and aromaticity. The presence/nature of the humic acids and the degradation products observed may be investigated in future work by a series of techniques. HPLC coupled with diode array detection would provide non-destructive spectral infromation regarding the conjugation of such products. Mass spectrometric detection would be greatly beneficial for molecular mass information and molecular constituent fragmentation information. Without such expensive apparatus, isolation could be achieved by collecting the fraction eluted by HPLC at the time of detection of the degradation product. A series of large volume injections could yield enough pure product to be dried and perform I.R. or NMR to further elucidate its structural characteristics.

It has been found that uptake of 1-naphthol on wet soil is due to hydrophobic sorption [10]. Wetting the soil will influence the degree and type of sorption due to factors such as water solubility of applied chemical and waters effects on soil properties. Wet soil is less adhesive than dry soil and in soils with high moisture contents, the water will compete for sites with organic compounds. Hydrophobic interactions between the phenols and the soil would be expected to increase in the presence of water due to the 'like-dissolves-like' phenomenon. Therefore, in a saturated system we would expect to observe a pronounced increase of hydrophobic sorption of 1-naphthol compared to phenol due to phenols greater affinity to form H-bonds with water (increased solubility in water) and 1-naphthols affinity to

partition to a greater extent into hydrophobic phases. Based on this hypothesis, the following chapter is an investigation into correlations between % organic carbon/matter (and other soil properties) and the degree of partitioning of phenol and 1-naphthol into saturated soils.

5.5 References

1. British Gas Method for *'The Determination of Phenols in Soils'*; issued for use by Northumbrian water (Analytical and Environmental Services) by P. Knowles, Method no. **024** (1995) 1.
2. L.S. Lee, C.A. Bellin, R. Pinal and P.S.C. Rao, *Environ. Sci. Technol.*, **27** (1993) 165.
3. M.L. Brusseau, A.L. Wood and P.S.C. Rao, *Environ. Sci. Technol.*, **25** (1991) 903.
4. D.H. Freeman and L.S. Cheung, *Science,* **214** (1981) 790.
5. K.Othmers, Encyclopedia of chemical technology, **8** (1993) 108.
6. R. Calvet and M. Leistra, In *'Interactions between herbicides and the soil'*. R.J. Hance Ed.; Academic Press, London. **Chap. 1 & 2** (1980) 1.
7. S.W. Karickhoff and K.R. Morris, *Environ. Toxicol. Chem.*, **4** (1985) 469.
8. G.W. Bailey and J.L. White, *Residue review,* **32** (1970) 29.
9. M. M. Mortland, J.J. Fripiat, J. Chaussidon and J. Uytterhoeven, *J. Phys. Chem.*, **67** (1963) 248.
10. J.J. Hassett, W.L. Banwart, S.G. Wood, and J.C. Means, *Soil Sci. Soc. Am. J.*, **45** (1981) 38.

11. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 160.

12. I.S. Butler and J.F. Harod, In '*Inorganic Chemistry Principals and applications*'; Benjamin/Cummings Publishing company, Inc. Redwood City, California, (1989) p277.

13. E. Suess, *Geochim. Cosmochim. Acta*, **37** (1973) 2435.

14. Y-P. Chin and W.J. Weber, Jr., *Environ. Sci. Technol.*, **23** (1989) 978.

15. R. Kretzschmar, W. P. Robarge and A. Amoozegar, *Water Resour. Res.*, **31** (1995) 435.

16. D. Grolimund, M. Botkovec, K. Barmettler and H. Sticker, *Environ. Sci. Technol.*, **30** (1996) 3118.

17. R.A. Wisdom, P. Dunnill and M.D. Lilly, *Enzyme Microb. Technol.*, **6** (1984) 443.

18. A.G. Livingston and H.A. Chase, *Chem. Eng. J.*, **45** (1991) b35.

Chapter 6.0

Partitioning of radiolabelled phenol and 1-naphthol in soil

6.1 Background to partitioning

6.1.1 Introduction

The transport through soil horizons and ultimate disposition of pollutants in ground water systems can be affected by biological activity, catalysis, sorption, desorption and partitioning processes between aqueous and soil phases. For example, water soluble humic acids can form micelles similar to those found with surfactants which can partition chemicals such as 1-naphthol in their hydrophobic interior [1]. Humic acids can also complex with 1-naphthol which decreases sorption to soil surfaces and increases transport rates in field soils [2]. Burgos et al. [3] proposed 2 mechanisms for the irreversible binding of 1-naphthol in wetted sandy soils. Firstly, biologically mediated oxidative coupling which is an important microbial synthesis route for phenolics to form humic substances in soil. The second is catalytic oxidation of phenolics by metal oxides [4]. The resulting oxidised organic may be a free radical or phenoxonium ion, but either species can form covalent linkages with the soil humic substances through an oxidative coupling reaction. The modeling of these processes for assessment of risk or evaluation of decontamination alternatives must include quantification of these processes.

6.1.2 Sorption and desorption

Common sorption mechanisms include physical sorption, hydrogen bonding (H-bonding) and chemical sorption [5]. Such sorption mechanisms can occur on a well defined soil surface or to amorphous structures such as soil organic matter [6]. The forces involved in physisorption are collectively termed 'van der Waals'. Van der Waals forces

are short range interactions which decay rapidly with distance. These are a summation of dipole-dipole interactions, dipole-induced dipole interactions, induced dipole-induced dipole interactions and ion dipole interactions. Chemical sorption usually involves a high activation energy and is exothermic due to strong specific chemical bond formation to attain a low energy stable state. The phenomenon of H-bonding lies somewhere between physical sorption and chemical sorption. Loudon [7] describes H-bonding as being comprised of two factors. Firstly a weak covalent interaction between a hydrogen on the donor atom and unshared electron pairs on an acceptor atom of another molecule. Secondly an electrostatic attraction between oppositely charged ends of two dipoles.

In a soil/solution system, on sorption of one large molecule, there is a displacement of water held in structured hydration shells around the molecule and the soil surface. In this manner, sorption can be favoured by the entropy gain to the system [8-9]. However, water sorbed to the surfaces of soil pores has a higher viscosity than normal water and is ice-like in nature [10]. This can result in mass transfer limitations which restrict solute diffusion and sorption, for example, phenol has been shown to compete with water as a H-donor or acceptor in the clay interlayers of montmorrilonite [11].

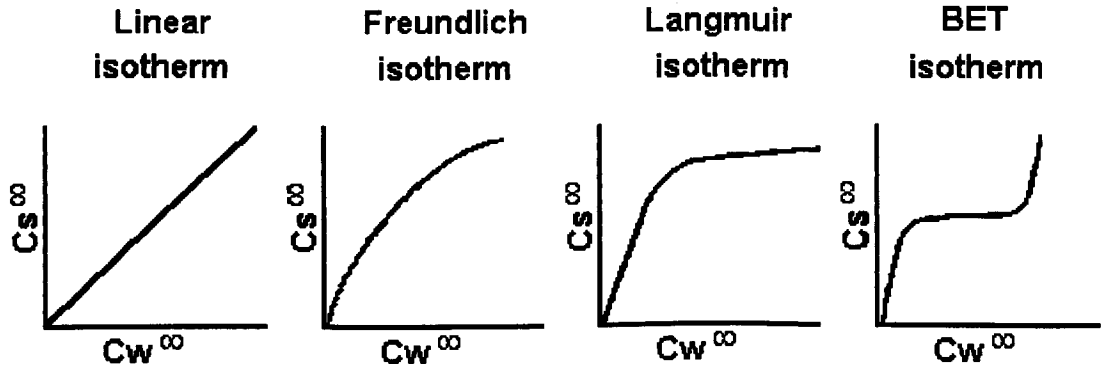
Desorption is generally slower than sorption and requires energy for release [9, 12]. The kinetic energy of desorption (Edes*) is the sum of the thermodynamic energy of sorption 'Q' (i.e. the depth of the potential energy well) and the activation energy of sorption (Eads*) [13].

6.1.3 Isotherms

A sorption isotherm shows the relation between the amount of analyte sorbed to a matrix and its bulk phase concentration, at equilibrium, at constant temperature. Data from isotherms can be used to correlate the extent of binding of an analyte by a soil with the soil's properties. This information may in turn be used to predict the fate of a chemical applied to a known system. Figure 6.1 highlights four commonly found isotherms [14]:

Figure 6.1

Examples of sorption isotherms



Where Cs^{∞} = amount sorbed at equilibrium (moles/kg), Cw^{∞} = solution phase concentration at equilibrium (moles/dm^3).

Each type of isotherm can pertain to a different sorption mechanism which is dependent on both the soil and analyte properties. According to the kinetic theory of sorption developed by Giles et al. [15] the slope of the Langmuir isotherm (or the extra amount sorbed per unit increase in equilibrium solution concentration), falls as more

solute is sorbed because it becomes more difficult for the analyte to find vacant sites on the sorbent as sorption proceeds (i.e. the maximum sorption possible is that of a complete monolayer). The Langmuir equation is only useful when multilayer sorption or catalytic condensation phenomena are not involved and implies that the heat of sorption is independent of surface coverage assuming a uniform surface [5].

The slope of the BET isotherm at first increases then levels when a monolayer forms followed by a further increase because sorbed molecules help to retain additional sorptive (i.e. multilayer formation), but eventually space considerations limit the extent of sorption. In one case, Giles et al. [16] ascribe the inflection on these isotherms to reorientation of aromatic molecules at the surface.

The 2 most common types of sorption observed in 'batch experiments' with soil are described by the linear and Freundlich isotherms. The equations describing equilibrium sorption for these isotherms are :

linear $\quad Cs^{\infty} = Kp\, Cw^{\infty}$ (eqn. 6.1)

Freundlich $\quad Cs^{\infty} = Kd\, Cw^{\infty\,(1/n)}$ (eqn. 6.2)

Taking logs of both sides of (6.2) we get :

$$\log Cs^{\infty} = \log Kd + n \log Cw^{\infty} \qquad \text{(eqn. 6.3)}$$

Kd and n can be obtained by linear regression. n is an exponent reflecting the curvature of the isotherm and may be taken to represent the energy distribution of

sorption sites [6]. Kp & Kd are partition & distribution coefficients. Kp (partitioning - equation 6.1) describes a linear relationship between concentration bound and concentration free due to equal sorption site energies on/in a homogenous surface/phase. On the other hand, Kd (distribution - equation 6.2) describes non-linearity due to a distribution of sorption energies on/in a heterogenous surface/phase. Equation (6.1) is derived from (6.2) where n = unity.

Linear sorption isotherms are often observed for narrow concentration ranges and low concentration levels and are indicative of constant partitioning into a homogenous phase. If sorption is controlled by hydrophobic partitioning to an organic phase this isotherm is thermodynamically predicted to be linear over a wide concentration range approaching solubility [17-18]. Researchers have noted many cases of linearity for non-polar organics sorption in soils, sediments and aquifer materials [19-22]. This is consistent with the partitioning mechanism of non-polar compounds into soil organic matter [23-24].

Non-linearity frequently occurs when large concentration ranges are used and sorption becomes more dependent on concentration. This is often a characteristic of a sorption processes arising from site specific interactions on a heterogenous surface where the affinity of a solute decreases progressively with increased concentration as sites become filled [9, 25]. Recent studies have shown that some organic compounds with polar functional groups are observed to sorb non-linearly [22, 26-27].

If sufficient time is allowed, and biological activity is controlled, sorption/desorption equilibrium should eventually be attained in soil/solute systems in the

laboratory [28]. In a fully reversible sorption system where sorption is controlled by weak forces, sorption and desorption isotherms should have the same partition coefficient. When an analyte is at sorption equilibrium in a closed system between a soil and aqueous phase, if a volume of the aqueous phase is removed for analysis, the equilibrium is disturbed. In accordance with 'Le Chatelier's' principle, when the volume removed is replaced with an equal volume of analyte free solvent, the sorption equilibrium alters to maintain the initial equilibrium (concentration ratio). This means that a portion of the bound analyte must be released from the solid matrix to maintain the ratio of free and bound analyte at equilibrium. Therefore, when sorption and desorption are at equilibrium the slope of the isotherms and hence Kd or Kp, should be the same. However, desorption isotherms frequently show kinetic 'hysteresis' which is the name given to a desorption isotherm which follows a different pattern to the sorption isotherm. A hysteresis is thought to occur due to the kinetics of desorption proceeding more slowly than those for sorption [29-31]. This may be due to diffusion limitations by the pore structure of soil (plus additional interactions with the soil during desorption) and modification of the soil during the shaking period. Desorption is also an endothermic process and the higher activation energies associated with analyte release (i.e. Edes* > Eads*) also contribute to lengthy desorption equilibration [32]. The desorption isotherm has a higher Kd when it occurs above the sorption isotherm, and a lower Kd when it lies below the sorption isotherm.

When the desorption isotherm possesses a higher Kd than the sorption isotherm this can be interpreted to mean;

- That sorption/desorption equilibrium has not been reached in the time frame (i.e. that either further sorption is occurring in the desorption step or that desorption is rate limited).
- That sorption is fully reversible.

When the desorption isotherm possesses the same Kd as the sorption isotherm;

- Sorption is reversible to a level governed by the Kd and equilibrium is attained in the time frame of the studies.

When the desorption isotherm possesses a lower Kd than the sorption isotherm;

- Sorption is reversible to a level governed by the Kd and further desorption occurs due to loss of the retention properties of the soil.
- Dilution in the desorption step favours the release of phenol (or 1-naphthol) due to competition by water molecules for sorption sites. Therefore the soil/water ratio is a critical factor governing sorption and desorption.

6.1.4 Predicting partitioning

'Partitioning' of an analyte into the organic matter of a soil (primarily by physical sorption) is a particularly popular mechanism covered in the literature [33]. Its commonality is of key importance in fate mechanisms since organic matter contains humic and fulvic acids which are water soluble and can transport bound chemicals over large distances in the soil profile. Partitioning (Kp or Kd) is often found to increase in a linear fashion as a soils % organic matter increases. The assumption that partitioning is

solely due to % organic matter allows simple predictions to be made as to the fate of chemicals in the environment.

The partition coefficients for a given 'nonionic' organic chemical are often normalised by accounting for the organic carbon fraction of the sorbent to give a characteristic constant, Koc (organic carbon referenced partition coefficient) [34-36].

$$Koc = Kd/foc \qquad \text{(eqn. 6.4)}$$

Where foc is the fractional organic carbon content of soil or sorbent on a mass basis. Therefore, knowledge of the Koc value of an analyte and the foc of a soil allows prediction of the Kd. Furthermore, the degree of hydrophobic sorption (Koc) has been predicted from octanol-water partition coefficients (Kow) and water solubilities (S) of analytes following the linear free energy relationship below;

$$\log Koc = a \log Kow \text{ (or } S) + b \qquad \text{(eqn. 6.5)}$$

Prediction of Koc from this equation can be used to predict the sorption uptake (Kd) for soils from equation 6.4. However, these equations assume the uniformity of sorption capacity and composition of organic matter regardless of its origin and mode of transformation. As a result, the partitioning with organic carbon or organic matter may not necessarily be described solely by the quantity of these phases. In a series of recent experiments, Xing et al. [29, 37], Chen et al. [38] and Jazen et al. [39] studied the sorption of radiolabelled 1-naphthol onto organic sorbents varying in polarity and aromaticity by 'batch equilibration (see 6.1.5). The results of these investigations [29, 37-

39] showed that Koc was not constant for a series of soils with varying % organic matter as assumed by equations 6.4 and 6.5. Koc of 1-naphthol was found to increase linearly as the 'aromaticity' of organic matter increased and that the aromaticity of humic acids in soils and sediments increased with soil depth.

6.1.5 Batch experiments

Sorption isotherms are commonly measured with batchwise experiments [28]. The procedure essentially involves the addition of a series of concentrations of an analyte in solution to a solid matrix. The analyte is allowed to interact with the matrix until equilibrium is reached. As previously discussed, the extent of interaction may or may not be concentration dependent. When equilibrium is reached, the sorbent is separated from the aqueous phase by centrifuging. An aliquot of the supernatant is filtered so that the equilibrium concentrations in the aqueous phase can be measured. The amount sorbed is calculated by difference. The desorption step involves replacing the volume removed in the sorption step with analyte free solvent and the system is allowed to re-equilibrate. This is again followed by centrifuging, filtration and analysis to find the desorption isotherm. It has been common in many experiments to falsely conclude that a system is in equilibrium after 1 or 2 days, and there is growing evidence to suggest these time scales may be unrealistic and batch studies may require longer to reach equilibrium [25, 40-45].

The parameters used in batch sorption methodologies and collection of isotherm data vary a great deal in the literature [25, 29-30, 46-51]. These procedures differ in bottle volume, buffer and electrolyte application, bacterial inhibition, equilibration time, measuring procedure, concentration range and soil/solution ratio. Ultimately, procedural

variations can lead to inconsistencies in literature values for sorption and confusion over different conclusions being made about the environmental fate of the same chemical. For example, Xing et al. [29] found a small amount of sorption of phenol on goethite from aqueous solution whilst Yost and Anderson [52] found no sorption of phenol. Similarly, Zhang et al. [53] found considerable sorption of phenol on montmorillonite whilst Xing et al. [29] found no sorption attributed to montmorillonite. As equilibrium time in a saturated soil is related to the soil's properties, it is not uncommon to find different times for sorption equilibrium of the same analyte. For example, the sorption of phenol on different soils has been observed to take between 5 hours [54] and 2 days [29]. Also, Hasset et al. [34] found that equilibration time for naphthol in batch systems was < 24 hours, whilst Chen et al. [38] used equilibration times of 2 days and have shown that 1-naphthol doesn't degrade in these systems in 2 weeks [37].

Research groups have measured sorption using soil/solution ratios ranging from 1:1 to 1:40 and higher [29, 34, 46, 54]. Excessive soil/solution ratios lead to unrealistic field estimates and coupled to extensive mixing can affect the physico-chemical properties of the matrix surface by causing increased particle dispersion and breakdown [32]. On the other hand small ratios may hinder desorption and lead to extended equilibration times in sorption experiments. Although increased solution volume may aid diffusion of analyte into soil pores, competition for sorption sites between water and compound as well as the solubility of the compound will affect the degree of sorption [8].

Soil/solution ratio should be kept as close to field conditions as possible to be able to draw any representative conclusions to fate mechanisms. It is surprising to note that most batchwise sorption studies in the literature do not involve an initial matrix wetting

step. The analyte solution is applied directly to dry soil and therefore may sorb analyte to a greater extent than saturated soil, for reasons previously mentioned. As the soil matrix in the field is generally saturated, application to dry soil will lead to unrealistic over-estimates of sorption. The same is true when a high solution/soil ratio exists and under-estimates may be found.

Sterilisation procedures are often used to inhibit bacterial degradation of the target analyte, so the final result can be attributed to the soil properties and not its microflora. Sterilisation procedures include treatment with NaN_2, $HgCl_2$, autoclaving and gamma irradiation [49, 54-55]. However some studies have shown that soil sterilisation techniques (such as autoclaving) can effect sorption in ways that could invalidate direct comparison with field estimates by altering the soil structure [56-57].

Batch equilibration systems commonly include an electrolyte such as $CaCl_2$, the concentration of which varies in the literature [2, 29]. However, Chen et al. [2] have shown that Ca^{2+} (from $CaCl_2$) binds strongly to the carboxylic groups of humic acids causing precipitation through salt formation. Humic acid flocculation in the presence of $CaCl_2$ was found to be synchronous with 1-naphthol release. A buffer is also generally added to maintain the pH of batch systems. When the analyte under investigation is ionisable, a pH is usually chosen to maintain the analyte in its neutral form. For example Xing et al. [29] used a pH 7 buffer when analysing phenol by the batch technique. At this pH phenol is below its pKa of 9.98 and in its unionised form [29]. Controlling the pH and the addition of an electrolyte is another way in which results become invalid to a realistic environmental situation. These conditions may lead to the alteration of the chemical

properties (i.e. buffering capacity) of the soil system and can therefore invalidate sorption measurements.

6.2 Experimental

6.2.1 Introduction

Batch equilibration isotherms were used to examine the effects of the soil properties measured (chapter 4.0), on the sorption of phenol and 1-naphthol. Due to the low concentrations used in these experiments LSS detection was used. This method of detection requires the radioisotopic analogues of the analytes studied. Radioactive (^{14}C) phenol and 1-naphthol were supplied in sealed glass vials by 'Sigma' dissolved in 100 µl toluene with 1.85 x 10^6 Bq and 3.7 x 10^6 Bq of activity, respectively. The toluene was evaporated by mild heating under a nitrogen flow and the residues transferred to separate 500 ml amber volumetric flasks with distilled water, made up to the mark and kept at 4 °C in the fridge. Inactive stock solutions of phenol and 1-naphthol were also prepared in distilled water and stored at 4 °C.

6.2.2 Equilibration time of phenol on garden soil

Primarily, the sorption equilibration time of phenol was determined on garden soil. The laboratory temperature and hence isotherm temperature was generally stable at 19 °C. 'Garden soil water' solution was prepared by adding 150 ml of distilled water to 50 g of garden soil. The soil/solution mixture was stored in sealed glass jars in the dark at ambient temperature and shaken up once a day for one week before the experiment so

that the soil/solution pH could equilibrate. After this time the soil/solution supernatant was centrifuged and filtered by buchner filtration prior to use in the experiments to remove any particulates which would contribute to sorption. The 'soil solution' was used to wet the soil instead of an 'artificial' pH buffer/electrolyte system, since it will contain extractives that, when added to dry soil will afford the minimum disturbance to the soils pH and other chemical characteristics. This procedure also has the advantage of producing information which is more reliable to field estimations.

Before addition of the soil solution to the individual soils, $HgCl_2$ was added to give a concentration of 10^{-5} moles/dm^3 (i.e. 0.0004 g /150 ml) to minimise biological activity. Garden soil was weighed (c.a. 1 g) into a series of 10 ml glass centrifuge tubes and wetted with 1 ml 'soil water' then sealed with rubber bungs. The tubes were agitated for 2 days on a rotating disc (Warburg mixer) at 32 revolutions/min and kept in the dark to hinder photodegradation. The wetting period was required to saturate the dry soil matrix to a degree indicative of field conditions. This saturation activates soil surfaces in a similar way to column activation in HPLC or sorbent activation in SPE.

A standard concentration of active + inactive phenol (641 nmoles/dm^3 + 2731 nmoles/dm^3) was added to all the tubes in 2 ml aliquots. After this addition of phenol the final soil/solution ratio was 1:3. The tubes were again rotated and sampled at times over a 41 day period in duplicate (last point in triplicate). A series of controls and a blank were also included and sampled in duplicate at each time point. The blank contained soil and water whist the control contained the same amount of active (and inactive) phenol as the samples but without soil present. The control was used as an external standard by which all measurements could be calculated with respect to 100 % recovery. The control was

also representative of any sample loss by partitioning of phenol to the headspace or sorption to the glass tube or rubber bung. At each sampling time, control, sample and blank tubes were taken from the Warburg mixer and centrifuged for 20 min at 3000 rpm. A 1 ml aliquot of the supernatant solution was removed from each tube and filtered through a 0.45 μm membrane Acrodisk into 5 ml 'pony' scintillation vials. 1.5 ml of liquid scintillation cocktail was added to each solution before sealing the vials. Because of the low energies of β-particles and short ranges in matter, the most feasible way to detect these radionuclides is to dissolve them in a liquid scintillator. When β-particles are released from radioactive phenol or 1-naphthol into the liquid scintillant, light is emitted and detected. The intensity of the light depends on the concentration of β-particles present and hence the frequency of decay or emission. The solutions were thoroughly mixed by vibration and placed in the autosampler of the LSS before each sample was analysed for radioactivity. The LSS measured decays per min (DPM) over a 20 min time period per sample and generated a report of the average DPM value. The liquid scintillation spectrometer contained internal calibration solutions which ensured linearity of the response as a function of concentration. The results obtained from LSS related DPM to concentration and were used to calculate the bound and free concentrations in the sorption step. The difference between the initial and final concentrations of free phenol were attributed to sorption/partitioning onto the soils.

To monitor the disturbance to the soils physical nature over long term equilibration, scanning electron micrograph's (SEM's) were taken of dry soil and treated (wetted & shaken) soil at 25 days and 41 days.

The repeatability of the technique was measured by analysing multiple controls filtered from the same stock solution.

6.2.3 Sorption/desorption of phenol and 1-naphthol on soils

From the results of the initial experiment, 2 days was assumed to be the time for phenol and 1-naphthol to reach sorption equilibrium on all the soils. 2 days was also assumed as the desorption equilibration time. These time scales are also commonly found to be applied in literature batch studies with phenol and 1-naphthol [29, 38].

The batch method used for sorption/desorption experiments was applied to all the soils described in chapter 3 (except site 3). Soil water solutions were prepared for the individual soils studied as described previously. These were used to wet the soils for 2 days prior to analyte addition. Batch equilibration isotherms were determined for phenol and 1-naphthol with initial concentrations between 641 - 10,396 nmoles/dm^3 (table 6.1) and 519 - 6985 nmoles/dm^3, respectively. These concentrations are well below the solubilities of these compounds in water (0.9925 moles/dm^3 for phenol [58] and 0.00601 moles/dm^3 for 1-naphthol [34]). All analyte solutions were prepared in distilled water by adding a constant amount of active (labelled) phenol/1-naphthol to a varying amount of inactive phenol/1-naphthol. Adding this mixture to the soil eliminated favoured sorption of either active or inactive phenol. 2 ml of each mixture were added to the soils after the 2 day wetting period. Table 6.1 on the following page shows the concentrations used for phenol.

Table 6.1

Phenol concentrations added to each soil

Tube	Concentration Active nmoles/dm^3	Concentration Inactive nmoles/dm^3	Total phenol concentration added nmoles/dm^3
1	641	0	641
2	641	697	1338
3	641	1394	2035
4	641	2090	2731
5	641	2787	3428
6	641	3484	4125
7	641	4181	4822
8	641	4878	5519
9	641	5574	6215
10	641	6271	6912
11	641	6968	7609
12	641	7665	8306
13	641	8362	9003
14	641	9059	9699
15	641	9755	10396
Control	641	9755	10396
Blank	0	0	0

Following the addition of the analyte to each soil, the tubes were shaken for 2 days to reach equilibrium. After this time 1 ml was removed from the tubes and analysed as described. Single-step desorption was used to construct desorption isotherms. This was achieved by adding 1 ml of soil water to the tubes in order to replace the supernatant removed in the previous step. This allowed the system to re-equilibrate under the initial soil/solution ratio of 1:3. The tubes were vibrated and thoroughly shaken to break up the soil cake caused by centrifuging. Since desorption equilibration time was assumed to be equal to sorption equilibration time, the tubes were shaken for a further 2 days on the rotating disc. After this time they were centrifuged and 1 ml was taken from the supernatant, filtered, and analysed by LSS.

All glassware was extensively cleaned between experiments to assure no radioactive or particle carryover. At the end of each experiment test tubes containing soil

were filled with water and vibrated then rinsed to remove the soil cake which forms after centrifugation. Tubes were then filled with methanol and sonicated to remove any particulate matter. Finally tubes were soaked overnight in 10 % HNO_3 (v/v) followed by rinsing with excess water and drying in a thermostated oven at c.a. 100 °C.

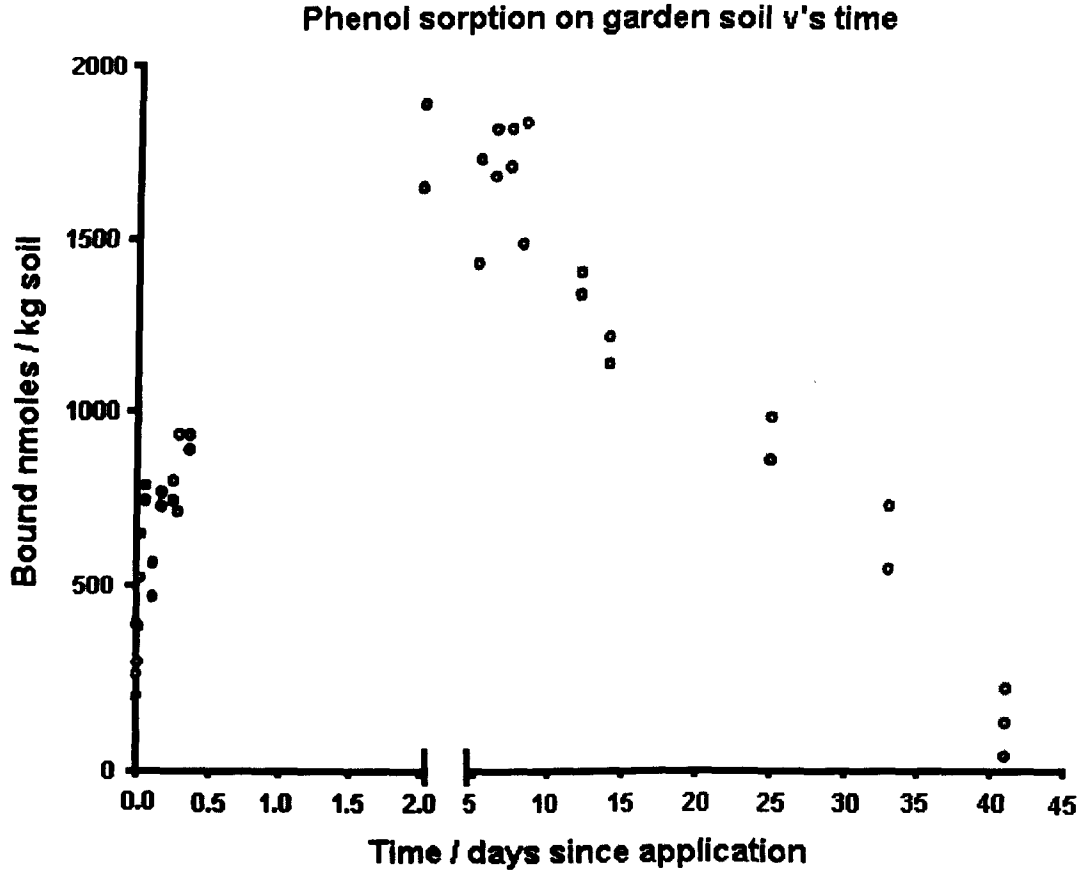
Isotherms for sorption and desorption of phenol and 1-naphthol were plotted as amount sorbed nmoles/kg soil versus concentration of free nmoles/dm^3. The partition coefficients were calculated from the isotherms generated. Both the Kd's and the isotherm type were interpreted and correlations with soil characteristics were attempted. Mathematical models were taken from literature references based on equation 6.5. The feasability of these models as predictive tools for the Kd (and Koc) values generated in these experiments was tested. The fate of phenol and 1-naphthol in these soils is briefly discussed in order to provide a bench mark for risk assessment.

6.3 Results

6.3.1 Equilibration time of phenol on garden soil

Figure 6.2 shows the concentration of phenol sorbed from solution to garden soil over 41 days:

Figure 6.2



Scanning electron micrograph's (SEM's) for untreated dry soil (figure 6.3) and treated (wetted & shaken) soil at 25 days (figure 6.4) and 41 days (figure 6.5) are shown on the following pages. These allow visible comparison of the physical changes in particle size and particle surface during the sorption study.

Soil particles under low magnification

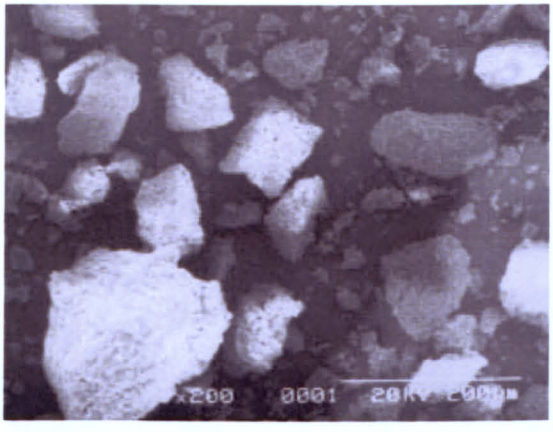


Figure 6.3

Dry sieved and ground soil

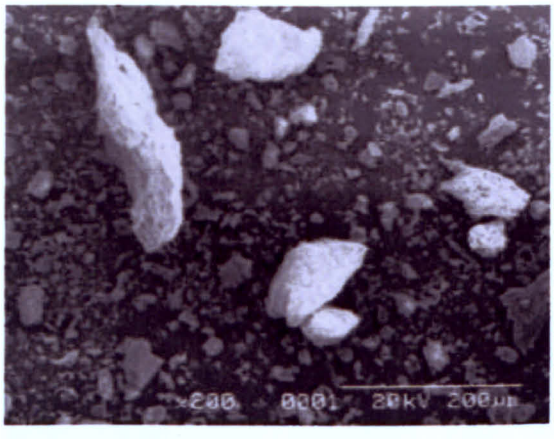


Figure 6.4

Soil wetted and shaken for 25 days

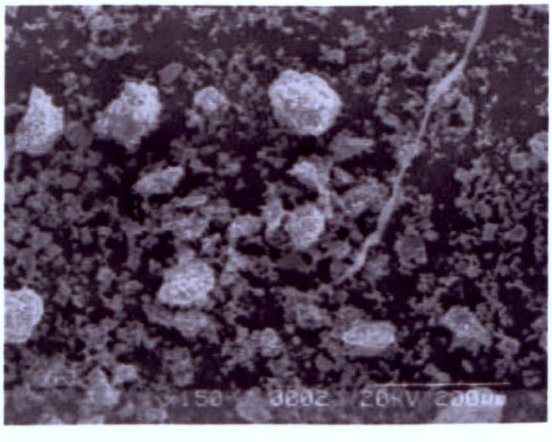


Figure 6.5

Soil wetted and shaken for 41 days

Soil particles under high magnification

Figure 6.3

Dry sieved and ground soil

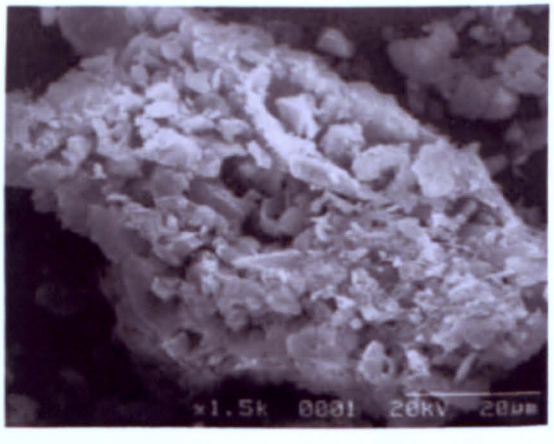


Figure 6.4

Soil wetted and shaken for 25 days

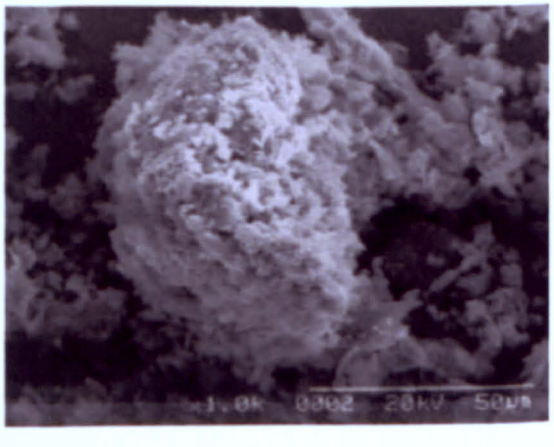


Figure 6.5

Soil wetted and shaken for 41 days

The repeatability of the measurement of radioactivity in control solutions (641 nmoles/dm^3 active phenol) by LSS gave a % RSD = 7.75 (n = 18).

6.3.2 Sorption/desorption of phenol and 1-naphthol on soils

The background radioactivity due to the blank was comparable in all the soils types studied and was found to be insignificant (< 1 %) in comparison to the lowest concentrations measured. The sorption/desorption isotherms for phenol and 1-naphthol included linear, Freundlich (non-linear), Langmuir and BET characteristics which are summarised in table 6.2 below. In cases where linearity is not observed the interpretation is more open to speculation.

Table 6.2

Type of sorption isotherm for phenol and 1-naphthol on the soils studied

		Sorption isotherm	
		Phenol	**1-naphthol**
Site1	**H/Ah**	Linear	Linear
	Ea	Linear	Non-linear/Langmuir
	Bhs	Non-linear	Non-linear
	C	Linear	Linear
Site 2	**Ah**	BET type	Non-linear/Langmuir
	Bg	Linear	Non-linear
	BCg	Non-linear	BET type
Site 4	**Ah**	Linear	Linear
	R	BET type	Non-linear/Langmuir
Garden soil		BET type	Linear

Figures 6.6 - 6.9 on the following pages are examples of each type of sorption/desorption isotherm. Further isotherms are given in Appendix B for phenol and 1-naphthol on the soils studied.

Figure 6.6

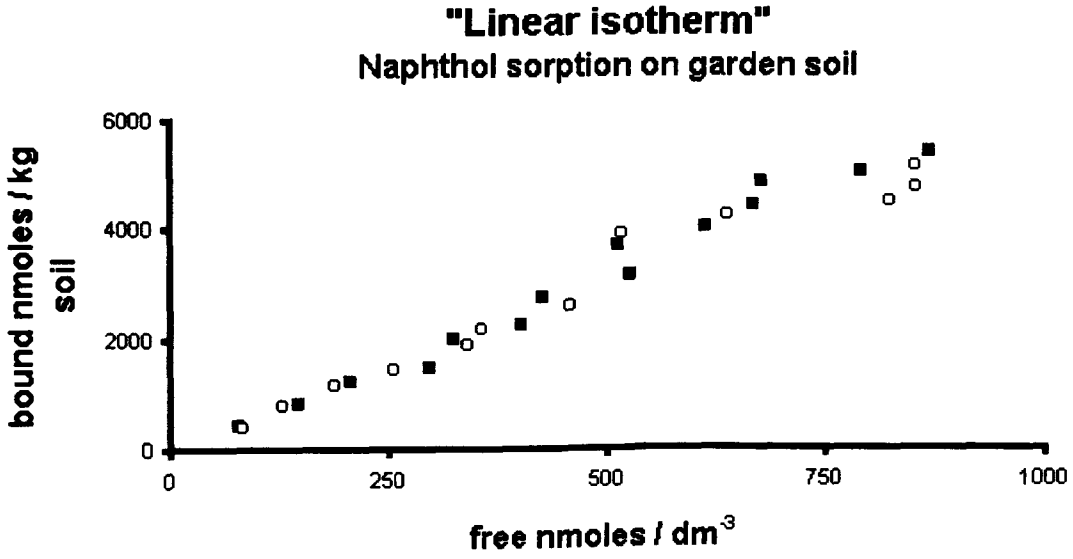


Figure 6.7

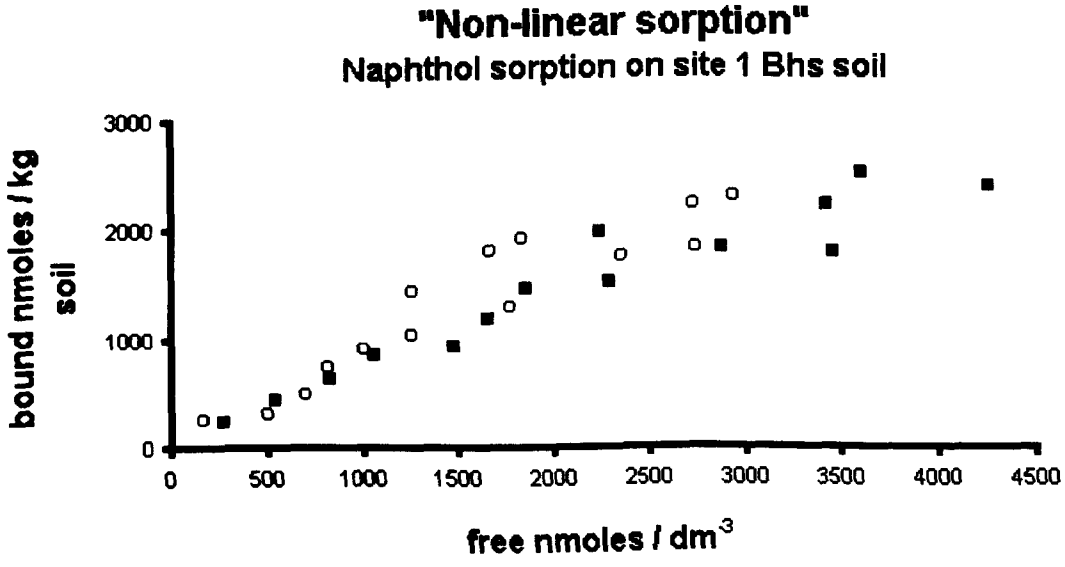


Figure 6.8

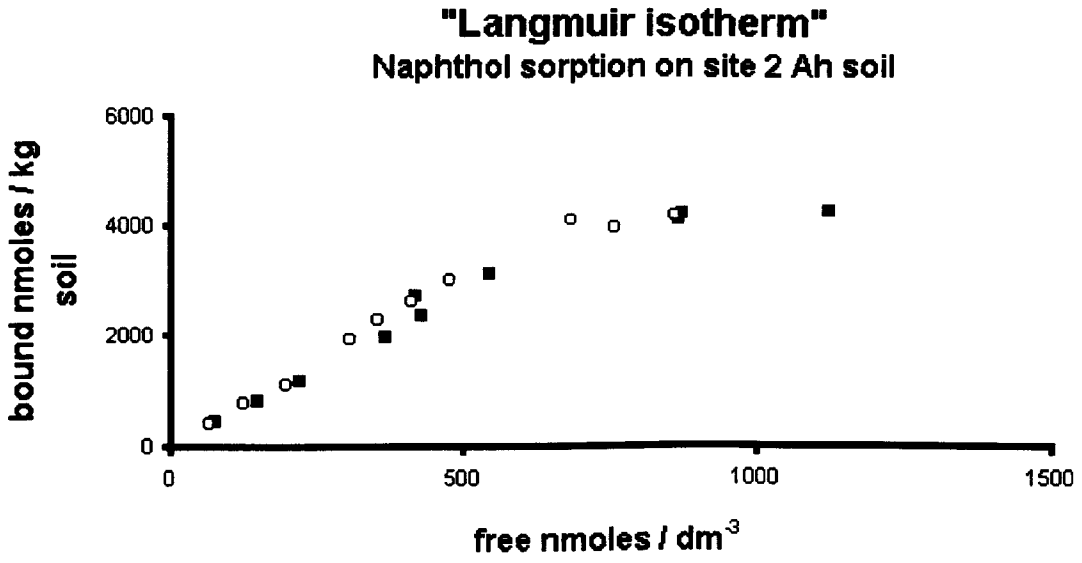
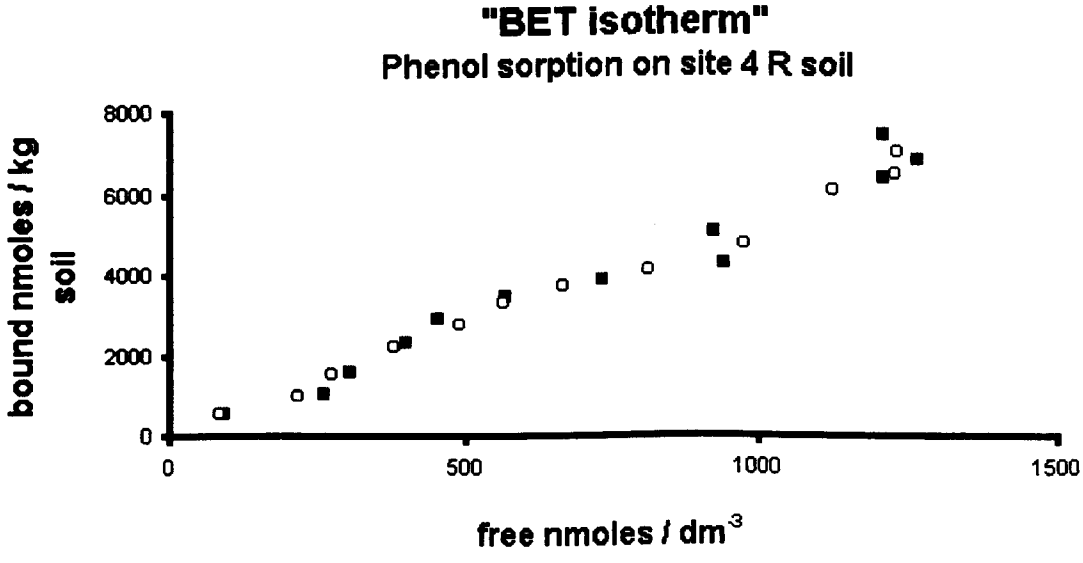


Figure 6.9



In comparison with sorption isotherms, the Kd's for desorption isotherms were equal in all cases except those shown in table 6.3.

Table 6.3

The type of desorption Kd's

		Desorption Isotherm Kd's	
		Phenol	1-naphthol
Site1	H/Ah	higher at high concentrations	lower at all concentrations
	Ea	lower at high concentrations	
Site 2	Ah	lower at all concentrations	
	Bg		higher at high concentrations
	BCg		higher at high concentrations
Site 4	Ah	lower at high concentrations	

6.3.3 Partition coefficients

For non-linear isotherms the sorption coefficient can be determined using either linear regression or the Freundlich model. Table 6.4 shows the comparison of sorption coefficients calculated using both the Freundlich and linear regression treatments. This highlights the effect of applying the Freundlich model to calculate Kd. As can be seen, Kd's are considerably larger when non-linearity is considered. Decreasing values for 1/n highlight the increased deviation from linearity.

Table 6.4

A comparison between linear and Freundlich partition coefficients

		Linear regression		Freundlich		
		Kd	R^2	Kd	R^2	1/n
phenol	Site 1 Bhs	3.39	0.97	6.55	0.97	0.93
	Site 2 BCg	3.76	0.82	9.7	0.94	0.88
1-naphthol	Site 1 Bhs	0.64	0.89	1.99	0.97	0.86
	Site 2 Bg	1.31	0.89	2.17	0.95	0.94

The partition coefficients for phenol and 1-naphthol on all the soils are shown in table 6.5 alongside the correlation coefficients (R^2) of the isotherms. Kd's for phenol and naphthol sorption on the soils possessing linear isotherms were calculated by linear regression. The Kd's of apparent BET isotherms were calculated by linear regression for simplicity, whilst only the linear portion of the Langmuir type isotherms were regressed. The Kd's of the non-linear isotherms were calculated using the Freundlich model to account for non-linearity. The fraction of organic carbon (foc) and % RSD's of each soil was found from the % organic carbon results in table 4.1. % RSD values for the scatter of each sorption isotherm are given next to each Kd value. Table 6.5 also shows the Koc values calculated for phenol and 1-naphthol using equation 6.4. The standard deviations for each Koc value were found using the following equations:

$$\text{Koc \% RSD} = \text{the square root of } (\text{Kd \% RSD}^2) + (\text{foc \% RSD}^2) \qquad \text{eqn. 6.6}$$

$$\text{Koc standard deviation} = (\text{\% RSD}/100) \times \text{Koc} \qquad \text{eqn. 6.7}$$

Table 6.5

Calculated Kd and Koc values for phenol and 1-naphthol on different soils

	Horizon	Phenol Kd (% RSD)	R^2	1-naphthol Kd (% RSD)	R^2	foc (% RSD)	Phenol Koc (+/- σ)	1-naphthol Koc (+/- σ)
Site1	H/Ah	2.93 (13.07)	0.94	2.99 (7.91)	0.98	0.3575 (4.16)	8.20 (1.10)	8.36 (0.72)
	Ea	0.78 (7.03)	0.97	0.69 (31.45)	0.94	0.0131 (5.92)	59.5 (5.5)	52.7 (16.6)
	Bhs	6.55 (14.65)	0.97	1.99 (15.68)	0.97	0.0164 (5.25)	399 (62)	121 (20)
	C	8.43 (9.80)	0.98	0.55 (12.73)	0.95	0.0117 (5.30)	718 (79)	46.8 (6.3)
Site 2	Ah	1.36 (11.65)	0.97	5.77 (13.96)	0.98	0.0658 (3.30)	20.6 (2.4)	87.6 (12.4)
	Bg	1.17 (12.22)	0.97	2.17 (17.84)	0.95	0.0273 (4.67)	42.8 (5.4)	79.4 (14.5)
	BCg	9.71 (22.23)	0.94	0.23 (26.92)	0.83	0.0092 (6.77)	1055 (245)	25 (6.9)
Site 4	Ah	1.47 (11.59)	0.95	3.58 (12.13)	0.96	0.0155 (7.20)	94.7 (12.8)	230 (32)
	R	5.46 (12.89)	0.96	2.65 (25.82)	0.95	0.0023 (11.42)	2333 (402)	1152 (325)
garden soil		1.89 (13.54)	0.94	6.39 (9.86)	0.98	0.0377 (1.45)	50.1 (6.8)	169 (16)

To asses whether any of the experimental Koc's can be predicted by partitioning of phenol or 1-naphthol between two homogenous phases (organic carbon/water), the equations below were employed from the literature.

- log Koc = log Kow - 0.317 [34] for poly nuclear aromatics (eqn. 6.8 'a')
- log Koc = 0.72 log Kow + 0.49 [35] for halogenated benzenes (eqn. 6.8 'b')
- log Koc = 0.989 log Kow - 0.346 [36] for poly nuclear aromatics (eqn. 6.8 'c')
- log Koc = -0.686 log S + 4.273 [34] for poly nuclear aromatics (eqn. 6.8 'd')

The results for the predicted Koc's from these equations are shown in table 6.6.

Table 6.6

Koc's for phenol and 1-naphthol predicted from solubility and Kow

	Phenol	1-naphthol
log Kow	1.46 [59]	2.9 [34]
log solubility	4.97 [58]	2.94 [34]
Koc calculated from (log solubility) [34]	7.3	181.1
Koc calculated from (log Kow) [34]	13.9	382.8
Koc calculated from (log Kow) [35]	34.8	378.4
Koc calculated from (log Kow) [36]	12.5	332.7

6.3.4 Correlating partitioning with soil properties

To assess whether any of the individual properties of the soils studied could be grouped together in order of the extent of partitioning, correlations were found between soil properties and partition coefficients of phenol and 1-naphthol. The values used are given in table 6.7.

Table 6.7

Soil properties and Kd values for phenol and 1-naphthol

Soils	Soil Properties									
	% moisture	Water capacity	% organic matter	% organic carbon	% carbonate	pH H2O	pH CaCl2	CEC	Kd	
									Phenol	Naphthol
Site1 H/Ah	8.33	60.69	48.31	35.75	4.54	3.94	2.97	89.82	2.93	2.99
Site1 Ea	0.14	17.27	2.32	1.31	5.22	4.41	3.51	1.53	0.78	0.69
Site1 Bhs	1.36	17.98	4.26	1.64	5.77	4.37	3.89	13.22	6.55	1.99
Site1 C	0.65	19.61	2.24	1.17	3.11	4.53	3.98	14.65	8.43	0.55
Site 2 Ah	1.96	28.31	13.6	6.58	7.43	5.7	4.61	24.8	1.36	5.77
Site 2 Bg	1.14	19.02	7.36	2.73	0.59	5.54	4.33	14.32	1.17	2.17
Site 2 BCg	0.88	21.26	4.50	0.92	0.54	5.10	4.14	10.57	9.71	0.23
Site 4 Ah	1.48	20.29	17.52	1.55	22.58	7.98	7.25	13.9	1.47	3.58
Site 4 R	0.11	15.35	17.18	0.23	21.43	8.57	7.61	3.36	5.46	2.65
Garden soil	1.86	23.34	10.44	3.77	5.99	6.61	6.09	24.79	1.89	6.39

The correlations between each of the soil properties and the Kd values are given in table 6.8. A scatterplot matrix (figure 6.10) is also given showing these correlations and the density elipses which encompas 95 % of the points.

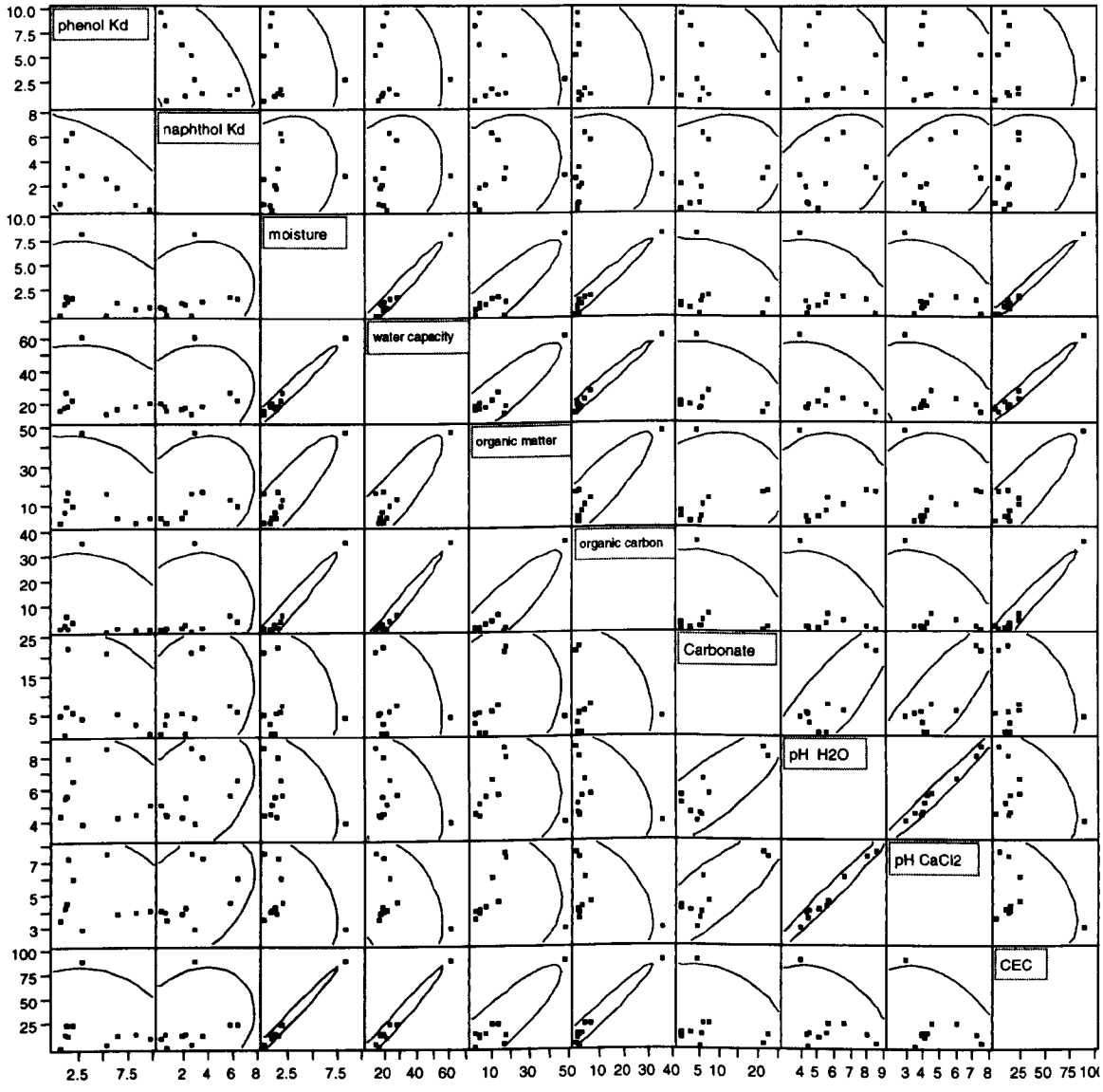
Table 6.8

Correlations between the soil properties and Kd values.

Variable	Phenol Kd	1-naphthol Kd	% moisture	Water capacity	% organic matter	% organic carbon	% carbonate	pH H_2O	pH $CaCl_2$	CEC
Phenol Kd	1	-0.5779	-0.1898	-0.17	-0.263	-0.1972	-0.2052	-0.1916	-0.1393	-0.1667
1-naphthol Kd	-0.5779	1	0.2482	0.2209	0.3134	0.1763	0.2839	0.4135	0.4186	0.2682
% moisture	-0.1898	0.2482	1	0.9854	0.9064	0.9847	-0.1614	-0.3618	-0.3818	0.9934
Water capacity	-0.1700	0.2209	0.9854	1	0.8953	0.9897	-0.1987	-0.392	-0.4233	0.9877
% organic matter	-0.263	0.3134	0.9064	0.8953	1	0.9109	0.217	0.009	-0.0321	0.8905
% organic carbon	-0.1972	0.1763	0.9847	0.9897	0.9109	1	-0.1759	-0.3923	-0.4254	0.9818
% carbonate	-0.2052	0.2839	-0.1614	-0.1987	0.217	-0.1759	1	0.8391	0.8488	-0.2002
pH H_2O	-0.1916	0.4135	-0.3618	-0.392	0.009	-0.3923	0.8391	1	0.9873	-0.3726
pH $CaCl_2$	-0.1393	0.4186	-0.3818	-0.4233	-0.0321	-0.4254	0.8488	0.9873	1	-0.3904
CEC	-0.1667	0.2682	0.9934	0.9877	0.8905	0.9818	-0.2002	-0.3726	-0.3904	1

Figure 6.10

Scatterplot matrix showing graphs between soil properties and Kd values



6.3.5 Pattern recognition results

To determine whether the soil properties can be used to group the soils together in relation to the Kd values measured for phenol and 1-naphthol multivariate techniques were used. The results from the correlation matrix (table 6.8) were analysed by principal component analysis. This statistical technique reduces the dimensionality by using linear

combinations of the soil properties (i.e. forces all the results into two dimensions) and yield 'Eigen vector values' and 'principle component values'. The first two principal component values for each soil are given in table 6.11. The first two principal component 'Eigen vector' values for each soil are given in table 6.12. In table 6.11, the first two principal components explain 83% of the variation as outlined by the cumulative % of the Eigen vector values in the second row of table 6.12. For simplicity, the characterisation values for soil pH measured in $CaCl_2$ solution (and not water) are chosen to represent pH in the results.

Table 6.11

The values of the first two principal components.

Soil	Principal component 1	Principal component 2
Site1 H/Ah	6.2611	0.3765
Site1 Ea	-0.8820	-1.3950
Site1 Bhs	-0.5327	-1.4046
Site1 C	-0.7434	-1.9938
Site 2 Ah	0.4951	0.8128
Site 2 Bg	-0.3932	-0.6565
Site 2 BCg	-0.7692	-2.0619
Site 4 Ah	-1.1779	2.6854
Site 4 R	-2.0693	2.2417
Garden soil	-0.1886	1.3953

Table 6.12

Principal Component Analysis showing Eigen vectors.

	Eigen vector 1	Eigen vector 2
Moisture	0.4267	0.0849
Water capacity	0.4292	0.0617
Organic matter	0.3654	0.2680
Organic carbon	0.4291	0.0627
Carbonate	-0.1356	0.4696
pH $CaCl_2$	-0.2326	0.4613
CEC	0.4273	0.0750
Phenol Kd	-0.0812	-0.2949
1-naphthol Kd	0.0761	0.4078
Total Eigen value	5.2791	3.0625
Percent	52.7911	30.6245
Cumulative %	52.7911	83.4157

The plot of the first principal component against the second is given in figure 6.11 and the plot of the first set of Eigen vector values against the second set of Eigen vector values is given in figure 6.12. Figure 6.11 shows the groupings of the soils by interrelated soil properties and partition coefficients whilst figure 6.12 shows groupings of interrelated soil properties and partition coefficients. By viewing figure 6.11 and 6.12 together it is possible to group soils together and determine which properties influence this grouping.

Figure 6.11

Principal component 2 by principal component 1

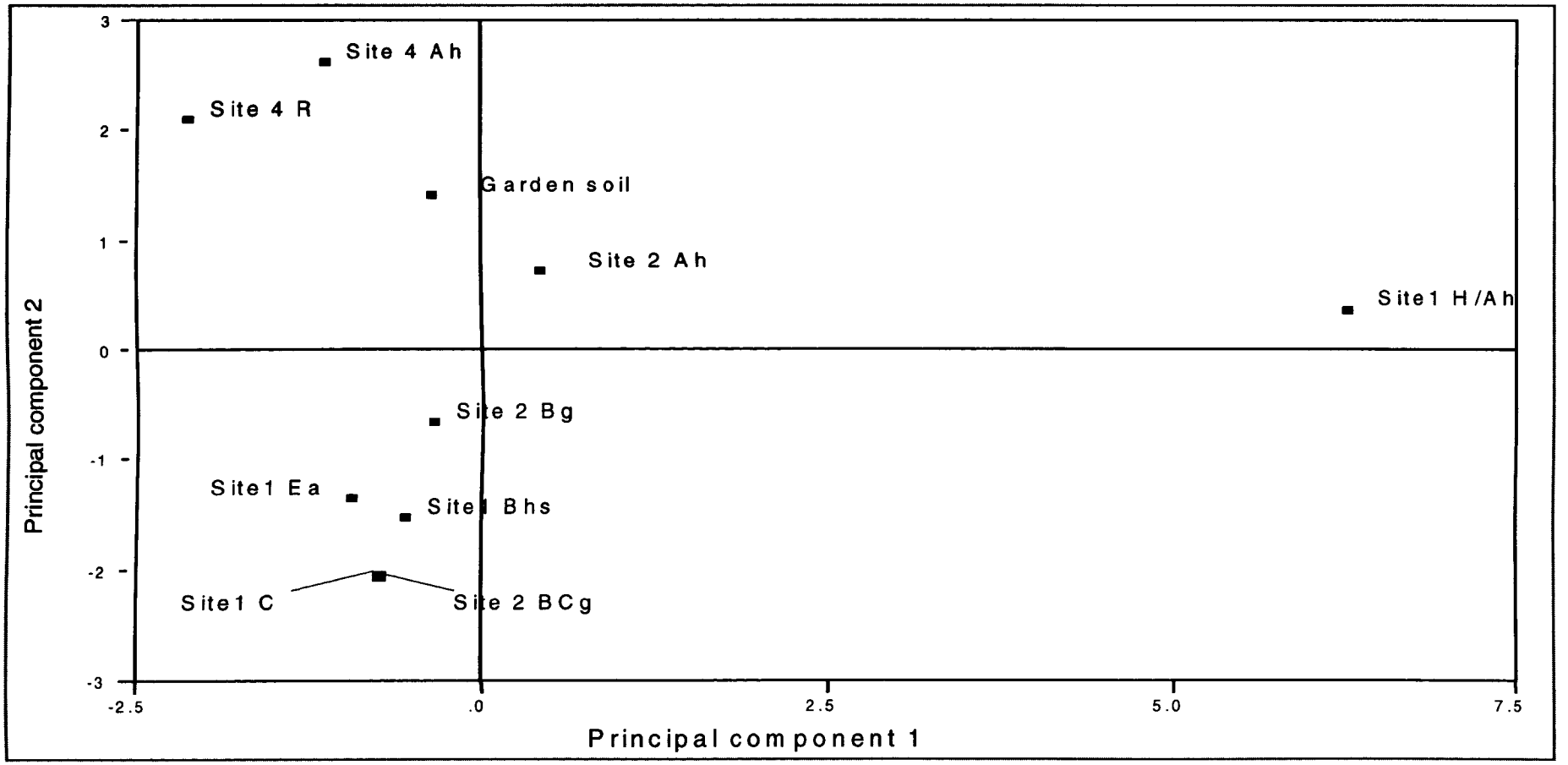
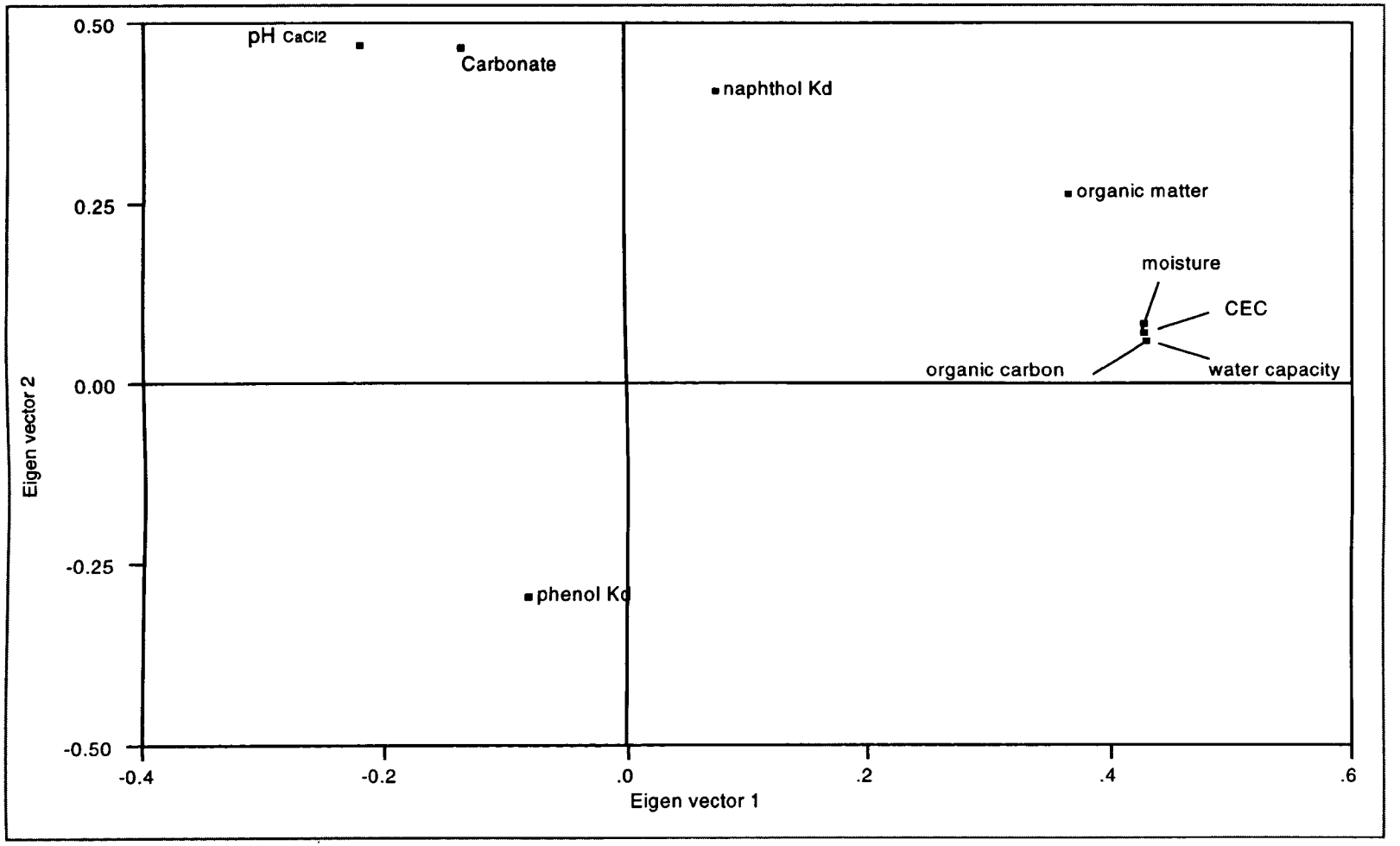


Figure 6.12

Eigen vector 2 by Eigen vector 1



6.4 Discussion

6.4.1 Equilibration time of phenol on garden soil

The results for the time analysis sorption study (fig. 6.2) suggest that an initial fast sorption step occurs, possibly as phenol is sorbed to specific high energy sites. Equilibrium is maintained within the system until day 8. This is followed by continual desorption of phenol back into solution. It was observed in the later extractions that the supernatant became slightly opaque with brown coloration. This colour passed through the 0.45 μm filter.

From figs. 6.3-6.5 it is evident that the act of continually rotating the wetted garden soil changes its physical characteristics. The particle size distribution is significantly more homogenous after prolonged shaking and wetting periods. Under magnification soil particles appear to be aggregates composed from colloidal plate-like structures. With untreated dry soil, these particles are tightly bound/associated. After wetting and shaking, the plates appear to loosen and sheer off or dissociate from their host particles to form finer debris. The procedure of rotating the soil will cause friction between soil particles and glassware (i.e. generating heat which may aid in desorption). This inevitably leads to the continuous formation of finer and finer colloidal particles. As mentioned in chapter 5.0, small colloidal particles can facilitate transport in the soil profile of tightly bound organic compounds. The wetting/shaking involved in these experiments may mimic adverse field conditions suggesting that phenol may be strongly bound to fine particles which can pass through the filter and therefore be detected as free phenol in the supernatant analysed.

6.4.2 Sorption/desorption isotherms

The fact that phenol doesn't always show the same type of isotherm as 1-naphthol on the same soil would indicate different sorption mechanisms to different sites in the soil matrix. The linear isotherms found are predicted for sorption/partitioning to a homogenous phase which in theory should be independent of concentration. The isotherm for phenol and 1-naphthol on site 1 H/Ah is one example of this where sorption occurs to constant energy sites in a homogenous phase. Linear isotherms may also be due to the sum of partitioning into several homogenous phases.

The non-linear isotherms observed are indicative of sorption to energetically diverse sites on a heterogeneous surface. Although non-linearity is commonly observed for compounds approaching their maximum solubility value, the concentrations used in this study are much lower than the aqueous solubilities of phenol and 1-naphthol. At low concentrations sorption is linear as low energy sorption sites are easily filled quickly. As the concentration increases, low energy sites become saturated and high energy sites are limiting to further sorption, but may become filled over prolonged equilibration times. Freundlich constants are higher than the linear regression constant of the same isotherm (table 6.4) due to the form of the equation (eqn. 6.2) correcting the constant to linearity. Therefore, for the Freundlich constant to be meaningful, it depends on the long term equilibration sorption to higher energy sites to occur, otherwise it is purely empirical.

The BET or S-shaped isotherms imply that successively sorbed molecules are co-operating in the sorption of further molecules (with an increased availability of sites as sorption proceeds) to form multilayers. Langmuir sorption isotherms are classical to

monolayer formation and indicative of homogenous energy sites on a uniform surface. This therefore suggests that sites 1 Ea, 2 Ah and 4 R in these studies have a limited sorption capacity towards 1-naphthol.

From table 6.3 the hysteresis for phenol desorption from site 1 H/Ah has a slightly higher Kd at higher concentrations than the sorption isotherm, and naphthol was higher for 2 Bg and 2 BCg at higher concentrations. This is evidence which indicates further sorption to the soil or slow desorption due to diffusive limitations. Alternatively, a fraction of the sorbate may be irreversibly held by the soil. In the case of site 2 Bg which has a non-linear sorption isotherm, the increased sorption at higher concentrations in the desorption step may be evidence for the slower equilibration in high energy sites described by the Freundlich constant.

Where the Kd for the hysteresis is lower than the sorption Kd this may be due to alterations in the physical properties of the soil aiding release of the sorbed molecules. This has previously been highlighted in the kinetic equilibration study of phenol sorption on garden soil. In the case of site 1 H/Ah, the increased apparent desorption for 1-naphthol detected in the supernatant may be due to increased extraction of soluble humic acids with bound 1-naphthol. Soluble humic acids are released from the soil matrix on extended water extraction and can pass through the filter as evidenced from the colouration of the supernatant in both the sorption and desorption steps. The fact that this doesn't occur with phenol in the desorption step would indicate that phenol is sorbed to the bulk solid.

6.4.3 Correlations between soil properties and partitioning

From the scatter plot matrix correlation's (figure 6.10) listed in table 6.8 no single characteristic is seen to govern the partitioning of phenol or 1-naphthol in all the soils. Therefore, these correlations are difficult to interpret with respect to any defined common contributions to sorption.

6.4.4 Principal component analysis

The results shown in figures 6.11 and 6.12 for the principal component analysis suggest that there are three soil groupings.

- Sites 4R, 4 Ah, 2Ah and garden soil.
- Sites 1Ea, 1 Bhs, 1 C, 2 Bg and 2 BCg.
- Site 1 H/Ah

Comparing the soil groups with the groupings in figure 6.12 it can be seen that the first group of soils (sites 4R, 4 Ah, 2Ah and garden soil) have a relationship between pH CaCl2, % carbonate and 1-naphthol Kd. The second group of soils (sites 1Ea, 1 Bhs, 1 C, 2 Bg and 2 BCg) have a positive relationship with phenol Kd. Soil 1 H/Ah has correlations with % moisture, water capacity, CEC, % organic carbon and % organic matter. Therefore the only group of soils to have correlation's between partitioning coefficients and soil properties are sites 4R, 4 Ah, 2Ah and garden soil. This suggests that 1-naphthol partitioning is influenced in these soils by soil water pH and % carbonate. The pH and % carbonate in these soils is relatively high compared to values in the other soils

which would therefore suggest that alkaline soils high in carbonate content can be correlated with the partitioning of 1-naphthol.

6.4.5 Experimental and predicted Koc's

Normalising the Kd results for the fraction of organic carbon to give Koc, shows that sorption/partitioning for phenol has an inverse relationship with % organic carbon in the horizons of each soil site. Although there are no linear correlation's between the Kd values and any of the soil properties measured, this normalised scale shows that sorption/partitioning of phenol into organic carbon does increase with soil depth. This supports the observations of Xing et al. [37] Chen et al. [38] and Jazen et al. [39] that partitioning depends largely on the aromaticity of the organic matter which has been found to increase with soil depth. If humic substances are more aromatic in the soils collected from lower horizons this would explain the low Koc's observed for phenol and 1-naphthol into the peaty top soil site 1 H/Ah, whereas high Koc's were obtained for phenol with soils at lower depths containing little organic matter or carbon. Therefore it is the quality and not necessarily the quantity of the organic matter/carbon which may be the deciding factor in sorption in these experiments. Assuming this is the case, then the high Koc's observed for phenol at soil depth also suggests that on wetting, more aromatic (less acidic/lower polarity) humic acids from deeper soils may not be extracted into solution due to a higher association with minerals and lower water solubility. However, the Koc's for 1-naphthol show fluctuating relationships with soil depth which don't account for the observations of Xing et al. and are more open to speculation.

The high organic matter peaty soil from site 1 (H/Ah) showed moderate sorption Kd's for phenol and 1-naphthol in comparison to the other soils studied. For example, there was higher sorption of phenol to site 1 H/Ah than the Ah horizons of sites 2 and 4 whilst 1-naphthol was sorbed to a greater extent on the Ah horizons. However, when the Kd is normalised for % organic carbon, a considerable decrease can be seen in the corrected sorption of phenol and 1-naphthol on site 1 H/Ah in accordance with the observations of Xing et al. [29, 37] Chen et al. [38] and Jazen et al. [39]. The results from table 6.5 show the lowest sorption for phenol and 1-naphthol on the peaty fraction of site 1 (H/Ah) when Kd is normalised for foc. The water holding capacity of all the soils after saturation for 2 days ranged from 15-28 % with the exception of site 1 H/Ah which absorbed 60 % of the 3 ml of water (chapter 4.0). This is not surprising since young peaty soils are lower in aromaticity, higher in acidity and polarity and as a result have a strong interaction with water and hence a high negative heat of wetting. This high association with water may explain the observed low Koc for phenol and 1-naphthol. Also, with increasing organic matter content a greater amount of organic carbon will be dissolved into the aqueous phase thus diminishing the sorption capacity of the bulk solid phase and increased binding to dissolved organic matter.

From table 6.6 it can be seen that due to the higher aromaticity of 1-naphthol and hence hydrophobicity, it has a higher predicted value of Koc than phenol. Comparing the experimental Koc's in table 6.5 with the predicted Koc's for phenol and 1-naphthol in table 6.6 it can be seen that there is a great difference between the values. Firstly the predicted values are significantly higher when applying log Kow compared to solubility. The standard deviations for Koc with the soils studied suggest that none of the

experimental values can be significantly predicted by using the Kow and solubility equations presented.

Surprisingly, Hasset et al. [34] concluded a 'good' agreement between experimental Koc values (328 - 905) and the Koc value calculated from the Kow for 1-naphthol (383) with ten out of sixteen different soils without mentioning standard deviations in results. However, Koc varied between 328 - 15618 for the 16 soils [34]. In comparison, the experimental values of Koc (table 6.5) for the ten soils studied in this investigation varied between 8 – 2333 for phenol and 8 – 1152 for 1-naphthol. This variation in experimental results firstly highlights the low correlation between sorption, and soil organic carbon content and secondly the limitations in applying Kow values to calculate Koc (and hence sorption coefficients).

In the case of the peaty top soil H/Ah, further attention is required when interpreting its observed Kd and Koc. The wetting of this soil took considerable time due to its high water holding capacity and the wetted soil appeared to swell and form a single mass rather than the finer particulates found with the other soils. The sorption of phenol and naphthol on site 1 H/Ah may be limited by long diffusion. The filtered supernatant solution was a yellow/brown colour indicating the presence of dissolved fulvic/humic substances. Radiation counts are assumed to be free phenol or 1-naphthol. However, due to the method of detection, these experiments may not draw a clear distinction for the binding mechanism in all the soils since the phenol/1-naphthol radiometrically detected, may be associated with matrix extractives.

Furthermore, the presence of a dissolved humic/fulvic acid-phenol/1-naphthol complex implies their transport or leaching capability in the environment [38, 60-62]. With site 1 H/Ah, the fact that phenol and 1-naphthol have very similar Kd's may not be a coincidence correlating to soil properties but due to colour quenching posing a fixed limit on detection. Quenching of the light emitted from the liquid scintillant (when β-particles are released from radioactive phenol or 1-naphthol) by the humic colour may result in free concentration underestimations and hence sorption overestimations.

6.5 Summary and conclusions

The majority of the sorption isotherms were linear. However some isotherms exhibited non-linearity suggesting sorption mechanisms other than partitioning. Assuming sorption is reversible, on dilution in the desorption step the higher ratio of water to analyte would aid release of phenol or 1-naphthol until equilibrium is reached again in accordance with Le Chatelier's principle. However, hysteresis were observed in some of the soils studied, which may indicate an alteration in the sorption properties of the soil.

From the scanning electron micrographs of garden soil particles (figures 6.3-6.5) it is evident that the method of dispersion may have an effect on the physical characteristics of the soil depending on soil type. For example, fine particles may have shorter diffusion paths and sorption to their surface rather than partitioning into bulky polymeric aggregates may cause faster sorption and desorption.

The literature is dominated with the observation that organic matter plays a major role in the sorption of hydrophobic organic compounds in soil [19, 63-66] with little

attention given towards the sorption of more polar compounds. Hasset et al. [34] concluded that both % organic carbon and % clay are two separate and deciding factors in the sorption of 1-naphthol. These studies have shown that the sorption of polar compounds does not correlate with organic matter or carbon content for a wide range of soils with varying characteristics, and may be a function of many soil characteristics. The complexity of the balance between the factors influencing sorption of the phenols studied is illustrated by the variation of the observed partition coefficients. These values are not consistent with any single soil characteristic measured.

As mentioned, in the literature partitioning has been correlated to % organic carbon content of the soil. However, considering phenol in these experiments, when the Kd is normalised for the fraction of organic carbon (Koc) the inverse relationship with % organic carbon is true. The distinction for 1-naphthol is less clear, perhaps due to its increased hydrophobicity and lower solubility approaching a cross over point between bond formation and pure partitioning.

Some factors to consider when carrying out further characterisation studies will be aromaticity of organic matter and the type of minerals present in the soils. CEC can be associated with organic matter and also clay minerals [5] so it may be necessary to determine the contributions from each. The pH of the soil solution determines the degree of dissociation or association of phenols which in turn will determine sorption to cations in clays since the energy of sorption may be very different between dissociated and associated forms. It has been found that the sorption in clay systems of acidic compounds is dependent on solution pH [5].

Other soil properties which may affect sorption such as % clay and type, particle density, surface area, and surface acidity should be characterised [67-68]. Type of cations present may affect the sorption through weak or strong bridging interactions. Soil/solution ratio could also be investigated to observe sorption capacity under different field saturations with implications on leachability.

These tests could be remodified to calculate Kd using SPME to extract 1-naphthol from the headspace or directly using membrane SPME (discussed in part B) in order to exclude the effect of matrix interferences such as humic acids. This would solely attribute sorption to the non-soluble fractions of the soil. SPME could be coupled to HPLC to allow simple quantitation without the requirement of derivatisation often associated with polar compounds in GC. SPME-HPLC would also be useful to discriminate between 1-naphthol and its degradation products which will both be detected by liquid scintillation counting. The preconcentrating factor inherent in SPME would also accommodate the low concentrations monitored in these experiments.

6.6 The fate of phenolic compounds in the environment

In conclusion to 'Part A' of this report, comparisons are made below between the two techniques employed.

6.6.1 Partitioning in saturated soil v's sorption in dry soil

When comparing the data for the ageing studies and radiolabelled studies there are limited correlations between the 2 techniques and the sorption of phenol and naphthol.

For example, in the ageing studies sites 1 Ea and 4 R show the maximum sorption of phenol whilst in the radiolabelled studies 1 Ea has the lowest sorption of phenol and site 4R has a medium sorption. Similarly site 2 BCg which shows the highest sorption in the Kd studies shows only low sorption in the ageing studies. The low relative sorption of 1-naphthol compared to phenol in the radiolabelled experiments also contradicts the findings of the ageing experiments.

The high amount of free naphthol in the batch experiments can be explained by the following;

- The mechanism for sorption in dry aged soil is very different to that in wet soils. It is well documented that wet soils are less adhesive that dry soils and therefore sorb to a lesser extent [8]. This is due to varying solubility as a function of moisture content and degree of competition of organic compounds for sorption sites at different moisture levels.

From the results presented in chapter 5.0 it is believed that the rapid application of the phenols on soil deposits the phenols on mineral and organic surfaces and in soil pores. Once the solvent evaporates there is limited movement of the phenols in the soils (except in the gas phase) and this allows longer 'relative' interaction times with the same sorption site leading to stronger and localised bonding. Surfaces bringing phenols into close contact may also, over a long period of time, catalyse polymerisation.

- Continuous rotation of wet soil in the batch experiment probably 'activates' organic matter and other surfaces. For example, in a saturated system the carboxyl,

hydroxyl, carbonyl and amino functional groups of humic substances allow these polymers to act a donors or acceptors in hydrogen bonding processes. The hydroxyl groups of phenols therefore increase their potential to interact in this way with soil organic matter. Water allows diffusion and H-bonding/partitioning to these 'activated' surfaces and many more interactions per unit time until favourable sorption sites are found. However, continuous rotation may also alter the soils physical properties to facilitate analyte release.

- The high concentration of free radiolabelled phenol and naphthol detected in the high % organic matter soil 1 H/Ah may be due to partitioning into soluble humic substances.

It is difficult to make a correlation between the soil properties of the soils studied and the observed sorption other than 1-naphthol sorption on a small group of soils. This suggests that unmeasured soil properties control the sorption of phenols, or that a cumulation of sorption interactions with various properties are present. In final conclusion, the location of a release of phenols will be the primary deciding factor into the possibilities of eventual or direct contact with ecosystems containing populations of plants and animals. The most effective barrier between such contact is soil and the properties of the soil, whilst release into an aquatic system will largely rely on the properties of sediments for mediation. In this situation, degradation by sediments (or photodegradation) to more toxic products is a direct problem. The environmental conditions at the time of spillage (i.e. wet or dry) will also play a major role in the different mechanisms of remediation of phenols in soil.

6.7 References

1. M.J. Morra, M.O. Corapcioglu, R.V. Wandruszka and D. Marshall, *Soil Sci. Soc. Am. J.*, **54** (1990) 1283.
2. S. Chen, W.P. Inskeep, S.A. Williams and P.R. Callis, *Soil Sci. Soc. Am. J.*, **56** (1992) 67.
3. W.D. Burgos, J.T. Novak, and D.F. Berry, *Environ. Sci. Technol.*, **30** (1996) 1205.
4. A.T. Stone, *Environ. Sci. Technol.*, **21** (1987) 979.
5. G.W. Bailey and J.L. White, *Residue review*, **32** (1970) 29.
6. B. Xing, J.J. Pignatello and B. Gigliotti, *Environ. Sci. Technol.*, **30** (1996) 2432.
7. G.M.Loudon, In '*Organic Chemistry*', 2nd ed. Benjamin Cummings Publishing Company, Inc. 1988, Menlo Park, California.
8. G.W. Bailey and J.L. White, *J. Agric. Food Chem.*, **12** (1964) 324.
9. J.J. Pignatello and B. Xing, *Environ. Sci. Technol.*, **30** (1996) 1.
10. P.F. Low, *Proc. Nat. Conf. Clays Clay minerals*, **8** (1960) 170.
11. S. Yariv, *Soil. Sci. Soc. Am. Proc.*, **39** (1975) 474.
12. P.J. Isaacson and C.R. Frink, *Environ. Sci. Technol.*, **18** (1984) 43.
13. A.W. Adamson, In '*The Physical Chemistry of surfaces*'; John Wiley & sons: New York, (1976).
14. R.W. Walters and R.G. Luthy, *Environ. Sci. Technol.*, **18** (1984) 395.
15. C.H. Giles, D. Smith and A. Huitson, *J. Colloid Interface Sci.*, **47** (1974) 755.
16. C.H. Giles, T.H. MacEwan, S.N. Nakhwa and D. Smith, *J. Chem. Soc.*, **11** (1960) 3973.
17. C.T. Chiou, L.J. Peters and V.H. Freed, *Science*, **206** (1979) 831.

18. H. Sontheimer, J.C. Grittendor and R.S. Summers, Activated carbon for water treatment; DBGLO-Forschungstelle: Karlsruhe, Germany (1989).

19. C.T. Chiou, P.E. Poter and D.W. Schmedding, *Environ. Sci. Technol.*, **17** (1983) 227.

20. S.W. Karickhoff, *Chemosphere*, **10** (1981) 833.

21. P.I. Flory, *Discuss. Faraday Soc.*, **49** (1970) 7.

22. P. Nkedi-Kizza, P.S.C. Rao and A.G. Hornsby, *Environ. Sci. Technol.*, **19** (1985) 975.

23. D.R. Garbarini and L.W. Lion, *Environ. Sci. Technol.*, **20** (1986) 1263.

24. P. Grathwohl, *Environ. Sci. Technol.*, **24** (1990) 1687.

25. W.J. Weber, Jr.; and C.T. Miller, *Water Res.*, **22** (1988) 457.

26. P.S.C. Rao, L.S. Lee and R. Pinal, *Environ. Sci. Technol.*, **24** (1990) 647.

27. A.T. Kan, G. Fu and M.B. Tomson, *Environ. Sci. Technol.*, **28** (1994) 859.

28. S. Burchill, M.H.B. Hayes and D.J. Greenland, '*The Chemistry of Soil Processes*'; D.J. Greenland and M.H.B. Hayes, Eds.; John Wiley & Sons Ltd., **Chap. 6** (1981) 221.

29. B. Xing, W.B. McGill and M.J. Dudas, *Environ. Sci. Technol.*, **28** (1994) 466.

30. C.T. Miller and J.A. Pedit, *Environ. Sci. Technol.*, **26** (1992) 1417.

31. J.J.T.I. Boesten and L.J.T. Van der pas, *Soil Sci.*, **146** (1988) 221.

32. R. Calvet and M. Leistra, In '*Interactions between herbicides and the soil*'; R.J. Hance, Ed.; Academic Press, London. **Chap. 1 & 2** (1980) 1.

33. F.D. Kopinke, J. Porschmann and U. Stottmeister, *Environ. Sci. Technol.*, **29** (1995) 941

34. J.J. Hassett, W.L. Banwart, S.G. Wood, and J.C. Means, *Soil Sci. Soc. Am. J.*, **45** (1981) 38.

35. L.B. Barber, E.M. Thurman and D.D. Runnells, *J. Contam. Hydrol.*, **9** (1992) 35.

36. S.W. Karickhoff, *Chemosphere*, **10** (1981) 833.

37. B. Xing, W.B. McGill and M.J. Dudas, *Chemosphere.*, **28** (1994) 145.

38. Z. Chen, B. Xing, W.B. McGill and M.J. Dudas, *Can. J. Soil Sci.*, **76** (1996) 513.

39. R.A. Janzen, B. Xing, C.C. Gomez and M.J. Salloum, *Soil Biol. Biochem.*, **28** (1996) 1089.

40. P.V. Roberts, M.N. Goltz and D.M. Mackay, *Water Resour. Res.*, **22** (1986) 2047.

41. E.M. Harrison and J.F. Barker, *J. Contam. Hydrol.*, **1** (1987) 349.

42. J.M. Bahr, *J. Contam. Hydrol.*, **4** (1989) 205.

43. R.S. Kerr, In-situ aquifer restoration of chlorinated aliphatics by methantrophic bacteria; EPA/600/2-89/033; US Environmental Protection Agency, Environmental Research laboratory, US Government Printing Office, Washington, DC, 1989.

44. S.W. Karickhoff and K.R. Morris, *Environ. Toxicol. Chem.*, **4** (1985) 469.

45. J.T. Coates and A.W.J. Elzerman, *J. Contam. Hydrol.*, **1** (1986) 191.

46. S.A. Boyd, *Soil Sci.*, **134** (1982) 337.

47. G.P. Curtis, P.V. Roberts and M. Reinhard, *Water Resour. Res.*, **22** (1986) 2059.

48. K. Schellenberg, C. Leuenberger and R.P. Schwarzenbach, *Environ. Sci. Technol.*, **18** (1984) 652.

49. D.F. Connaughton, J.R. Stedinger, L.W. Lion and M.L. Shuler, *Environ. Sci. Technol.*, **27** (1993) 2397.

50. R.P. Schwarzenbach and J. Westall, *Environ. Sci. Technol.*, **15** (1981) 1360.

51. K. Banerjee, P.N. Cheremisinoff and S.L. Cheng, *Environ. Sci. Technol.*, **29** (1995) 1243.

52. E.C. Yost and M.A. Anderson, *Environ. Sci. Technol.*, **18** (1984) 101.

53. Z.Z. Zhang, P.F. Low, J.H. Cushman and C.B. Roth, *Soil Sci. Soc. Am. J.*, **54** (1990) 59.

54. H.D. Scott, D.C. Wolf, and T.L. Lavy, *J. Environ. Qual.*, **11** (1982) 107.

55. W.P. Ball and P.V. Roberts, *Environ. Sci. Technol.*, **25** (1991) 1223.

56. P.O. Salonius, J.B. Robinson and F.E. Chase, *Plant and soil,* **27** (1967) 239.

57. H.D. Skipper and D.T. Westermann, *Soil Biol. Biochem.*, **5** (1973) 409.

58. V.D. Radisav, M.T. Suldan and R.D. Brenner, *Environ. Sci. Technol.*, **27** (1993) 2079.

59. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 160.

60. G. Caron, I.H. Suffet and T. Belton, *Chemosphere,* **14** (1985) 993.

61. R.R. Engebreson, T. Amos and R.V. Wandruszka, *Environ. Sci. Technol.*, **30** (1996) 990.

62. B.R. Puri. In '*Activated Carbon Sorption of Organics from the aqueous Phase*'; I.H. Suffet, M.J. McGuire, Eds.; Ann Arbor Science: Ann Arbor,; Vol 1, Chapter 17 (1980).

63. F.J. Stevenson, *J. Environ. Qual.*, **1** (1972) 333.

64. A. Khan, J.J. Hassett and W.L. Banwart, *Soil Sci.*, **128** (1979) 297.

65. D.R. Ghosh and T.M. Keinath, *Environmental progress,* **13** (1994) 51.

66. M.L. Machesky, *Environ. Sci. Technol.*, **27** (1993) 1182.

67. N. Sawatsky, Y. Feng and M.J. Dudas, *J. Contam. Hydrol.*, **27** (1997) 25.

68. S.G. Pavlostathis and G.N. Mathavan, *Environ. Technol.*, **13** (1992) 23.

Part B:

Determination of organic pollutants in environmental matrices using solid phase microextraction (SPME)

Methods were validated to analyse both liquid and solid samples for pollutants using solid phase microextraction (SPME). The usefulness of the technique was studied as applied to qualitative and quantitative aspects for the determination of extractives. Both practical and theoretical aspects were investigated to gain a wider understanding of the technique.

Initial experiments focused on the qualitative use of SPME coupled to GC-MS as a fast screening procedure for extractives, regardless of matrix type. A quantitative method was firstly applied in conjunction with the analysis and separation of chlorobenzenes in aqueous samples. Success in these experiments was followed with an evaluation into the possible application to quantify levels of chlorobenzenes and PAH's in solid samples. Several contaminated soils were analysed, alongside reference materials for validation of the optimised technique. Initial observations highlighted the dependence of hydrophobicity on the extraction of chemicals within a certain class. Experiments were therefore designed to monitor the effect that different conditions had on the extraction/partitioning within a class of chemicals.

Chapter 7.0

Theoretical aspects of SPME

7.1 Introduction

Solid phase microextraction is an equilibrium technique. As a result, many of the factors mentioned in the previous chapter also apply to SPME. The sorbent in SPME is a polymeric fibre coating into which analytes partition. Polyacrylate (PA) coated fibres and polydimethylsiloxane (PDMS) coated fibres were investigated in the SPME experiments in the following chapters.

Considering the fibre in a solution system, as soon as this sorbent is placed in contact with analytes, partitioning begins between the phases until an equilibrium is reached. With a fibre in solution the amount sorbed at equilibrium is directly inferred from the concentration in the fibre (solid phase). The PA coating is a solid polymer whilst PDMS is a liquid polymer and therefore PA fibres are more resilient to high temperatures [1].

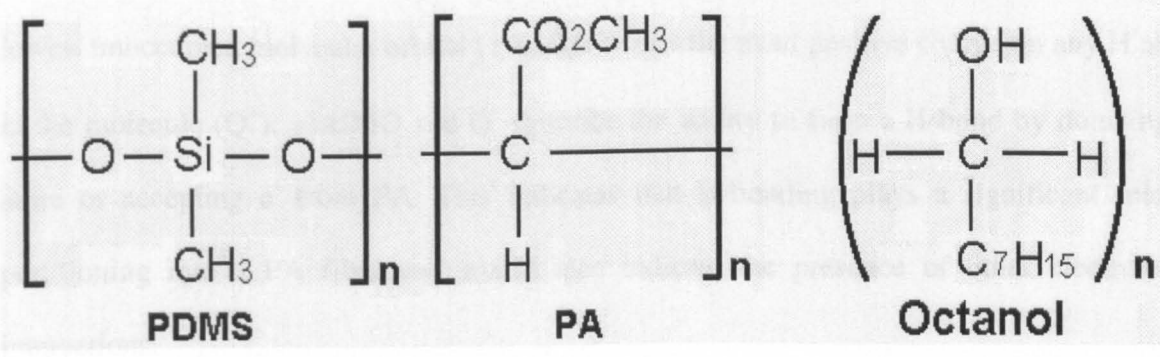
7.2 The influence of fibre coating properties on extraction

Kow is a universal constant used by many branches of science to relate a chemicals partitioning properties between water and a hydrophobic matrix/phase [2]. Octanol has a hydrophobic carbon chain and a polar hydroxyl group and as a result it can extract non-polar compounds and to a lesser extent polar compounds from aqueous samples and is commonly used as a measure of chemical partitioning in soils and living systems [2]. PA can extract both polar and non-polar compounds due to its hydrocarbon backbone coupled with relatively polar ester functional groups. On the other hand, the functionality of the PDMS

fibre is more non-polar than that of the PA fibre and partitioning has a higher dependence on polarity. Figure 7.1 shows a comparison of the structures of octanol, PA and PDMS.

Figure 7.1

The structures of octanol, polyacrylate and polydimethylsiloxane



The bonds in PDMS (i.e. Si-O and Si-C) have an asymmetric distribution of electrons (and hence a dipole moment). However, because the dipole moment is a vector quantity, symmetrical molecules with 'bond dipoles' have 0 polarity [3]. Therefore, due to the symmetry of the PDMS polymer it has no permanent dipole moment and is non-polar. Conversely, polyacrylate is polar since it has a permanent dipole moment. Each fibre has a large surface of electron clouds available for van der Waals interactions with analytes which include short lived attractive dipole induced dipole interactions which are the main contributing factor in partitioning of non-polar molecules [3]. The other force governing partitioning will be the electrostatic attraction of atoms in the molecule and fibre possessing different electronegativities. The most infamous electrostatic attraction is the 'hydrogen bond', the formation of which is favoured due to the lower electronegativity of hydrogen in comparison to other atoms present [3]. Therefore the increased polarity of PA will

undoubtedly contribute to the increased extraction of analytes via attraction by electrostatic forces.

Vaes et al. [4] employed multivariate techniques with physiochemical and quantum chemical descriptors to describe contributions to partitioning into the PA fibre. Several significant descriptors were found to affect sorption. These were Kow, the energy of the lowest unoccupied molecular orbital (ε LUMO) and the most positive charge on any H atom in the molecule (Q^+). εLUMO and Q^+ describe the ability to form a H-bond by donating H atom or accepting e^- from PA. This indicates that H-bonding plays a significant role in partitioning into a PA fibre and would also indicate the presence of other electrostatic interactions.

7.3 Extraction modes

There are 3 main sampling techniques in SPME

- Direct extraction of an aqueous or soil slurry sample
- Direct extraction of a sample using a membrane protected fibre
- Extraction of the headspace above a sample

Each technique involves 2 steps of fibre action using the fibre assembly described previously (chapter 2 figure 2.1).

(1) Exposing the fibre to the sample matrix to allow partitioning of the analytes between sample and fibre. The efficient isolation and extent of extraction and pre-concentration is dependent on several factors. These include exposure time, coating type and thickness, the partition coefficients of the analytes and their water solubility.

(2) The fibre is removed from the sample and introduced into the injection port of the GC where thermal desorption takes place at high temperature. To afford instantaneous injection by SPME the analytes are focused at low temperature at the head of the column. The focusing temperature is recommended as 70 - 90 ^{o}C (depending on column coating thickness) below the boiling point of the lowest boiling analyte [2]. Peak widths for a direct injected sample are about 20 seconds. Unlike direct injection, thermal desorption is not instantaneous and so peak widths would be significantly broader affecting sensitivity. Focusing is therefore required due to the time taken for thermal desorption [2].

7.3.1 Direct extraction of aqueous samples

In direct extraction, the fibre is immersed in the aqueous or soil slurry sample. Sorption of analytes by the fibre depends on their equilibrium fibre/solution partition coefficients Kfs (eqn. 7.1) which is equivalent to the soil/solution partition coefficient Kd or Kp determined in the previous chapter. Direct extraction is generally used for the efficient extraction of non-volatile/semi-volatile compounds which have high partition coefficients in the fibre. Kfs is given by equation 7.1 [2];

$$K_{fs} = C_f^{\infty} / C_s^{\infty} \qquad \text{(eqn. 7.1)}$$

Where C_f^{∞} is the amount sorbed at equilibrium and C_s^{∞} is the concentration (moles/dm^3) in solution at equilibrium. K_{fs} depends on the equilibrium affinity of an analyte for either the fibre or aqueous phase. The affinity for the aqueous phase usually depends on solubility or vapour pressure whilst chemical functionality determines interaction with the fibre coating used. Higher K_{fs} values reflect an increasing affinity of an analyte towards the fibre and hence increased sensitivity. However, analytes with high K_{fs} also have longer equilibration times if the matrix is not modified or agitated. Equilibration times can be decreased and sensitivity increased in several ways (see chapter 9 results and discussion). Experimentally, the amount sorbed by the fibre at equilibrium (C_f^{∞}) can be found using the equation 7.2 [2]:

$$C_f^{\infty} = C_s^{0} \times R \qquad \text{(eqn. 7.2)}$$

Where C_s^0 is the initial aqueous concentration (moles/dm^3) and R is a response factor. R is found from comparison between direct injection and SPME concentration versus area plots. For example, if the equation for a line (intercept = 0) for direct injection of 1 µl samples over a range of concentrations is y = 2000 x, and for SPME of 1 ml samples is y = 5000 x (where y is the peak integration area and x is the concentration). Accounting for the sample volume difference by multiplying the first equation by 1000 this gives the following equations :

$1000\,y = 2000000\,x$ for direct injection of 1 ml

$y = 5000\,x$ for SPME of 1 ml

The response factor is the ratio of the gradients (i.e. 5000/2000000 = 2.5 x 10^{-3}). Once C_f^{∞} is known for each analyte, Cs^{∞} and hence Kfs (eqm) can be calculated.

As C_f^{∞} is a maximum value for sorption at equilibrium, the sorbed concentrations at various times can also be found by multiplying C_A by the areas found at different times [2].

$$C_A = C_f^{\infty} / A^{\infty} \qquad \text{(eqn. 7.3)}$$

Where C_A is the sorbed mass (g) per unit area, and A^{∞} is the area at equilibrium.

In the following chapters where Kfs is calculated by direct extraction it is referred to as K_{fs}^{PA} when the polyacrylate coating is used and K_{fs}^{PDMS} when the polydimethylsiloxane coating is used.

The Kfs values calculated at equilibrium are normalised to volume unity (ml) for the fibre volume. This allows a direct comparison of sorption, dependent on coating type. The general equation which is used to calculate the normalised Kfs is shown below [2]:

$$K_{fs} = (C_f^{\infty} / Cs^0) \times (1 / V_f) \qquad \text{(eqn 7.4)}$$

Where C_f^{∞} is the amount sorbed at equilibrium, Cs^0 is the initial concentration and V_f is the volume of the fibre in cm^3 (0.000494 cm^3 for PA, 0.000628 cm^3 for PDMS). However, this equation is only used for analytes with low partition coefficients or when the sample volume is large and Cs^0 can be considered to be unchanged. The experiments in the following chapters employed small volumes of sample and the depletion of the analytes

(which had high partition coefficients) from the samples was large enough to effect Cs^0. Therefore the final value of K_{fs} was calculated from a modified form of equations 7.1 and 7.4 given by equation 7.5 below [2]:

$$K_{fs} = (Cf^{\infty} / Cs^{\infty}) \times (1 / Vf) \qquad \text{(eqn. 7.5)}$$

7.3.2 Direct extraction of a sample using a membrane protected fibre

Direct extraction with membrane protection is an alternative to direct extraction of non-volatile and semi-volatile compounds from matrices containing interferences detrimental to the performance of the fibre and the separation/detection system [2]. The extraction mechanism is basically the same as direct extraction with possibly even slower kinetics. The use of this technique is not common at present in the literature and won't be discussed further.

7.3.3 SPME extraction of the sample headspace

SPME has been applied to the extraction of analytes from the static headspace above the sample matrix [2]. Headspace extraction can be used to efficiently extract volatile analytes which have large solution/headspace partition coefficients. The kinetics for diffusion of an analyte into the fibre in the gas phase are more rapid than in solution. This results in faster attainment of equilibration which can be advantageous if selective extraction of volatiles from non-volatiles is required (see below).

As with direct extraction, sensitivity using headspace extraction is also dependent on the analytes partition coefficient into the fibre. The headspace is in thermodynamic equilibrium with the sample resulting in a 3 phase equilibrium between sample, headspace and fibre [2, 5-6]. The total amount of the analyte in the closed system should remain constant during extraction so the amount of analyte in each of the phases at equilibrium can be given by [2];

$$Cs^{0} Vs = Cs^{\infty} Vs + Ch^{\infty} Vh + Cf^{\infty} Vf \qquad \text{(eqn. 7.6)}$$

Where;

$Cs^{0} Vs$, is the initial (or total) concentration (Cs^{0}) in the solution volume (Vs)

$Cs^{\infty} Vs$, is the equilibrium concentration (Cs^{∞}) in the solution volume (Vs)

$Ch^{\infty} Vh$, is the equilibrium concentration (Ch^{∞}) in the headspace volume (Vh)

$Cf^{\infty} Vf$, is the equilibrium concentration (Cf^{∞}) in fibre coating volume (Vf)

Analytes must be transported from the matrix and through the air barrier before reaching the fibre and therefore must possess high Henry's constants.

A Henry's constant is basically a measure of volatility and can therefore be expressed as the solution/headspace partition coefficient Khs or Ch^{∞}/Cs^{∞}. Henry's constants are used to predict exchange rates of vapours across the air/water interface. Henry's constants influence the rate of evaporation of organic solutes from aqueous and solid matrices.

Many matrix interferences are complex molecules which have low Henry's constants and as a result cannot traverse the air barrier. Analysing the headspace and eliminating matrix interferences from detection thus allows enhanced sensitivity of the analytes of interest. For a liquid sample, the mass of analyte sorbed by the fibre in the headspace at equilibrium can be expressed by [2]:

$$C_f^{\infty} = \frac{C_s^0 V_f V_s K_{fh} K_{hs}}{K_{fh} K_{hs} V_f + K_{hs} V_h + V_s} \qquad \text{(eqn. 7.7)}$$

Where;

V_f, V_s, and V_h are the volumes of fibre coating, solution and headspace, respectively.

$K_{fh} = C_f^{\infty}/C_h^{\infty}$ = fibre/headspace partition coefficient

$K_{hs} = C_h^{\infty}/C_s^{\infty}$ = headspace/solution partition coefficient

C_f^{∞}, C_s^{∞}, and C_h^{∞} are the equilibrium concentrations of analyte in fibre coating, solution and headspace. This equation can be used to predict the amount extracted at equilibrium based on knowledge of the gas/liquid partition coefficient. Experimental determinations of the concentration extracted by the fibre are achieved by relating the response of the SPME extraction to the response from external calibration standards (as mentioned above). K_{fh} is large as no liquid is present to hinder diffusion into the fibre matrix in the gas phase. Enhancement of vapour phase partitioning (K_{hs}) can be achieved by heating as well as similar means used for direct analysis (see results and discussion, chapter 10.0).

In a closed system the amount of analyte extracted at equilibrium is independent of the location of the fibre. The same amount will be extracted if the fibre is placed directly in the solution or in the headspace. However, the amount extracted at 'time x' (before equilibrium) is dependent on fibre location and is a very useful means of selective extraction.

For example, consider that the fibre is placed in the headspace above a solution containing naphthalene, which possesses a high Henry's constant (44.6 $Pa.m^3.mol^{-1}$ [7]). Naphthalene will rapidly partition into the headspace and be freely available for equilibration with the fibre. Rapid extraction kinetics are therefore facilitated, and equilibrium is reached in a short time. If the fibre is introduced into the solution of the system the majority of naphthalene will be in the headspace. Partitioning of dissolved naphthalene into the fibre will deplete the solution concentration thus forcing the re-attainment of the equilibrium between headspace and solution. Naphthalene must therefore diffuse back into solution and into the fibre until a steady state is achieved. Diffusion of analytes in solution into the fibre is slower than analytes in the headspace which will also increase the equilibration time.

In the following chapters where K_{fh} is calculated by headspace extraction it is referred to as K_{fh}^{PA} when the polyacrylate coating is used and K_{fh}^{PDMS} when the polydimethylsiloxane coating is used.

7.4 Theoretical aspects of equilibration in SPME

On introducing the SPME fibre into an aqueous solution there is an initial rapid depletion of the analyte from the area surrounding the fibre. In a static system, the analyte is extracted from the aqueous matrix unaided by external forces such as agitation. The time required for an analyte to reach equilibrium is therefore limited by its diffusion through the ever increasing depleted static layer of water surrounding the fibre.

It has been suggested that the orientation of water molecules around non-polar organic compounds is more ordered than in the bulk solution. Although more structured, these molecules are continuously interacting with the bulk solution. Frank and Evans [8] described this phenomenon as early as 1945 and similar ordering of water close to hydrophobic surfaces in soil pores and clay systems has also been described to substantiate this [9]. This ordering of water at hydrophobic surfaces is known as hydrophobic hydration, or more attractively termed 'flickering iceberg' formation. The extent of the iceberg increases with the size of the solute molecule. By analogy, for larger molecules, or for example an SPME fibre, the surrounding static layer of water can be visualised as a 'flickering ice sheet'.

During sorption of organic compounds on the surface of macroreticular polymers, the 'icebergs' around the organic compound and polymer will break up with an accompanying entropy gain to the system. This entropy gain is the main driving force in physical sorption from aqueous solutions. It is for this reason that initial sorption rates from solution are so

rapid. Methods of shortening equilibration time include 'agitation' and 'matrix adjustment'. These methods are considered below.

7.4.1 Agitation

Some experiments have shown that through agitation it is possible to achieve a sufficiently small aqueous diffusion layer around the fibre to prevent aqueous diffusion being a limiting factor in sorption [4]. Motlagh and Pawliszyn [10], Buchholtz and Pawliszyn [11] and Louch et al. [12] found that elimination of the static water layer around the fibre can be overcome by sonication or with rapid stirring. Vaes et al. [4] found that with higher stirring rates partitioning was limited by internal resistance in the PA fibre coating. PDMS is a liquid hydrophobic polymer and as a result is expected to have high analyte diffusion rates into the fibre. On exposing this fibre to an aqueous solution of analytes, high diffusion rates will rapidly diminish the analytes surrounding the fibre. Conversely, polyacrylate is polar and more solid, suggesting slower diffusion from liquid into solid controlled by fibre resistance to diffusion.

Sonication is a powerful means of agitation and the high flux of power entering the solution causes increase in temperature and fast equilibrium due to destruction of the ice sheet. Motlagh and Pawliszyn [10] compared agitation techniques and concluded that the most effective is sonication with a flow through cell.

7.4.2 Matrix adjustment

The aqueous matrix can be adjusted in different ways to increase partitioning and recovery whilst speeding up time taken to reach equilibrium. These methods are briefly discussed below:

- Saturation with sodium chloride salt [13-14] aids uptake of analytes by the fibre. This salting out effect is caused by neutral molecules becoming insoluble as water molecules prefer to solvate the electrolyte ions [15]. Depending on the size and charge of ions they may cause freezing or saturation of the water nearest to them decreasing the solubility of other compounds in the system (salting out) due to the lower concentration of free water [8]. Adding NaCl to an aqueous matrix prior to headspace analysis can enhance the isolation of target analytes from water, but can also enhance non-target analytes. If NaCl is simply added indiscriminately, it will salt out even highly soluble chemicals, as is the case with phenols. This problem can be overcome if element specific detectors or MS with ion selected monitoring is used.

- pH adjustment of the sample matrix has also shown to improve recoveries of certain analytes by maintaining their neutral forms [16-18]. For example to selectively extract polar compounds, such as phenols, in a mixture with amines, the solution can be acidified so that the phenols are in their neutral form. Ions will not partition into the fibre and so at lower pH the amine cations will remain in solution. The converse will be to extract basic or neutral compounds. For example, to selectively extract PAH's from a mixture of phenols and PAH's,

raising the pH above the pKa of the phenols will deprotonate the phenols and so the PAH's will be selectively extracted.

- When analytes have low partition coefficients, or where selective extraction is required, derivatizing the sample [19-21] or doping the fibre with derivatizing agent can increase partitioning [21-22].

7.5 References

1. S.L. Chong, D. Wang, J.D. Hayes, B.W. Wilhite and A. Malik, *Anal. Chem.*, **69** (1997) 3889.
2. J. Pawliszyn, In *'Solid Phase Microextraction, Theory and Practice'*; Wiley - VCH, Inc. John Wiley & Sons, Inc., 605 Third Avenue, New York, NY,(1997).
3. G.M.Loudon, In *'Organic Chemistry'*, 2nd ed. Benjamin Cummings Publishing Company, Inc. 1988, Menlo Park, California.
4. W.H.J. Vaes, C. Hamwijk, E.U. Ramos, H.J.M. Verhaar and J.L.M. Hermens, *Anal. Chem.*, **68** (1996) 4458.
5. B. Kolb, *Applied Headspace Gas Chromatography*. Heyden, London, UK, (1980).
6. B. Macgillivray and J. Pawliszyn, *J. Chromatogr. Sci.*, **32** (1994) 317.
7. W.Y. Shiu and D. Mackay, *J. Chem. Eng. Data*, **42** (1997) 27.
8. H.S. Frank and M.W. Evans, *The Journal of Chemical Physics.*, **13** (1945) 507.
9. P.F. Low, Proc. Nat. Conf. Clays Clay Minerals, **8** (1960) 170.

10. S. Motlagh and J. Pawliszyn, *Anal. Chim. Acta,* **284** (1993) 265.

11. K.D. Buchholz and J. Pawllszyn, *Environ. Sci. Technol.,* **27** (1993) 2844.

12. D. Louch, S. Motlagh and J. Pawliszyn, *Anal. Chem.,* **64** (1992) 1187.

13. Z. Penton, SPME Varian Application Note **11**, (1996).

14. J.F. Elder and P.V. Dressler, *Environ. Pollut.,* **49** (1988) 117.

15. R. Fessenden and J. Fessenden, In '*Organic Laboratory Techniques*';Brooks/Cole Publishing: Monterey, CA, (1984).

16. U. Mingelgrin and Z. Gerstl, *J. Environ. Qual.,* **12** (1983) 1.

17. Z. Zhang and J. Pawliszyn, *J. High Resolut. Chrom.,* **16** (1993) 689.

18. SPME. Supelco Application notes: **11,6,17 and 56**, (1994).

19. E. Pocurull, M. Calull, R.M. Marce and F. Borrul, *Chromatographia,* **38** (1994) 579.

20. Y. Ohkura and H. Nohta, *Adv. Chromatogr.,* **29** (1989) 221.

21. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.,* **66** (1994) 160.

22. M.E. Cisper, W.L. Earl, N.S. Nogar and P.H. Hemberger, *Anal. Chem.,* **66** (1994) 1897.

Chapter 8.0

Qualitative aspects of SPME : Matrix screening

8.1 Introduction

The use of headspace SPME coupled with gas chromatography and mass spectral detection (GC-MS) as a simple screening tool to carry out fast qualitative analysis was evaluated. Primarily this afforded the opportunity to minimise the time taken to develop the method by coupling the extraction and preconcentration of analytes with separation and qualification in a few simple steps. Due to the nature of the matrices analysed the headspace mode was used to minimise matrix interferences and fibre damage.

A diverse range of chemicals were extracted, separated and detected from several different solid and liquid matrices. In this initial validation exercise, the same extraction conditions were chosen and used with all the matrices without any prior optimisation. The liquid matrices analysed included 'Robinsons' orange juice and 'Coca Cola'. Solid matrices included two reference materials known to be contaminated with chlorobenzenes and one reference material contaminated with PAH's. The extractives of three industrially contaminated soils were compared with garden soil which would be expected to contain minimum levels of pollutants (described in chapters 5 and 6). Three varieties of tobacco and a sample of diesel exhaust soot were also screened. Qualitative analysis was accomplished via the injection of standard solutions of chlorobenzenes and PAH's (structures given in chapter 1) to allow identification via mass spectral data and retention times. Chromatographic parameters were optimised to separate these analytes.

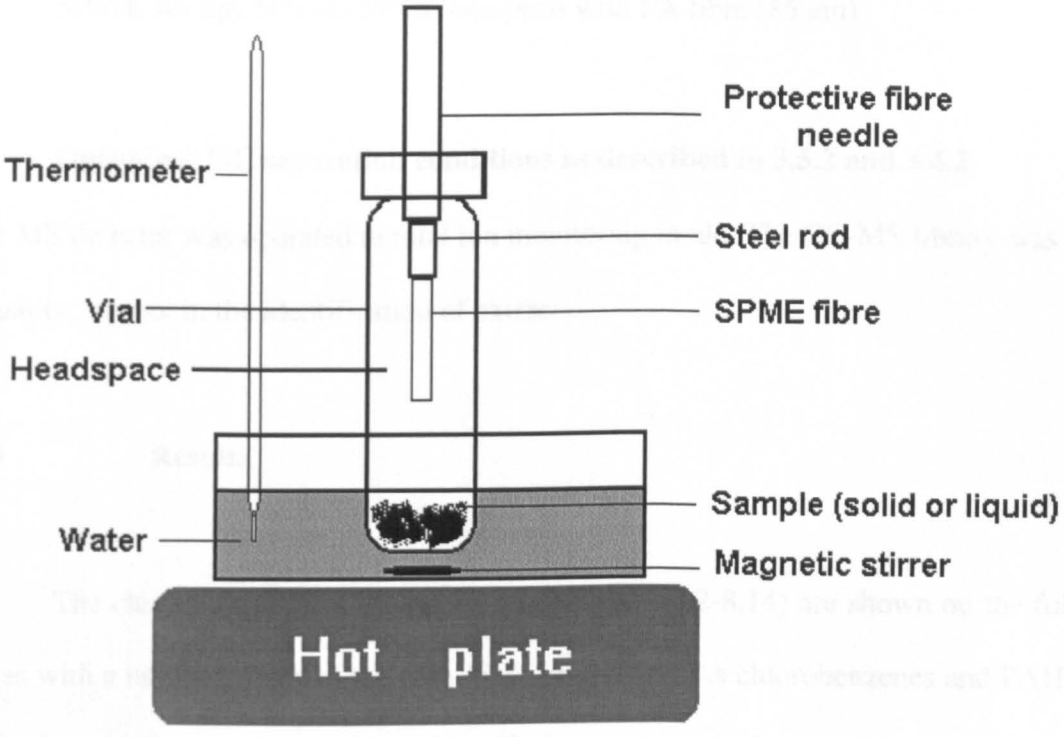
8.2 Experimental

Both liquid and solid matrices were heated in sealed vials to increase the partitioning of non-volatile compounds into the headspace. Heating was carried out using the apparatus in figure 8.1 on the following page. The temperature of the water bath was maintained at 85 °C in order to afford 'favourable' extraction conditions and the water was magnetically stirred for uniform distribution of heat. Both the manual SPME holder and the vial were held in place using a clamp stand (not shown).

Water was added to the solid samples to further facilitate release of analytes. Using the fibre set-up described in chapter 2, the fibre needle was used to pierce the septa of the vial and the fibre lowered into the headspace. After a fixed time the fibre was withdrawn into the needle, removed from the sample and transferred to the GC injection port. Here, analytes were desorbed from the fibre for 10 mins at 280 °C and the analyte vapour is held or 'focussed' at the head of the GC column at 50 °C to afford instantaneous injection. After 10 mins the fibre was 'regenerated' and removed ready for the next extraction. The GC temperature program given on the following page was used to separate the analytes extracted on the basis of their respective boiling points.

Figure 8.1.

SPME set up for matrix screening



The experimental conditions are given below for the different matrices;

- **Extraction conditions used for liquids**

  0.5 ml analysed in sealed 2 ml vial

  liquid heated to 85 °C

  10 min extraction time, 10 min desorption time

- **Extraction conditions used for solids**

  Between 0.02 and 0.2 g analysed in 2 ml vial

  0.5 ml water added

mixture heated to 85 °C

10 min extraction time, 10 min desorption time

- **SPME set up;** Manual SPME equipped with PA fibre (85 μm)

- **Optimised GC separation conditions as described in 3.5.2 and 3.4.2.**

The MS detector was operated in total ion monitoring mode. The GC-MS library was used as a qualitative tool in the identification of extracts.

8.3 Results

The chromatograms of the extractions (Figures 8.2-8.14) are shown on the following pages with a numbered key of the identified compounds. As chlorobenzenes and PAH's were the only analytes which were fully identified, analytes which were identified via the MS-library database are only named in the chromatograms where the quality of the mass spectra was greater than 90 %. Several common PAH's were found to be present in all the industrially contaminated soils, the LGC reference material, exhaust soot and Old Holborn tobacco. Flavour and fragrance compounds were identified in the foodstuffs and tobaccos. Comparison of the chromatograms of the tobaccos yielded very interesting information, concerning the observable extracted compounds, which relate directly to the different scents of each.

Figure 8.2

A GC-MS chromatogram of the SPME headspace extraction of 'Coca Cola'

1 = α-pinene, 2 = Limonene, 3 = Isoborneol, 4 = Borneol, 5 = Decanal, 6 = α bisabolol

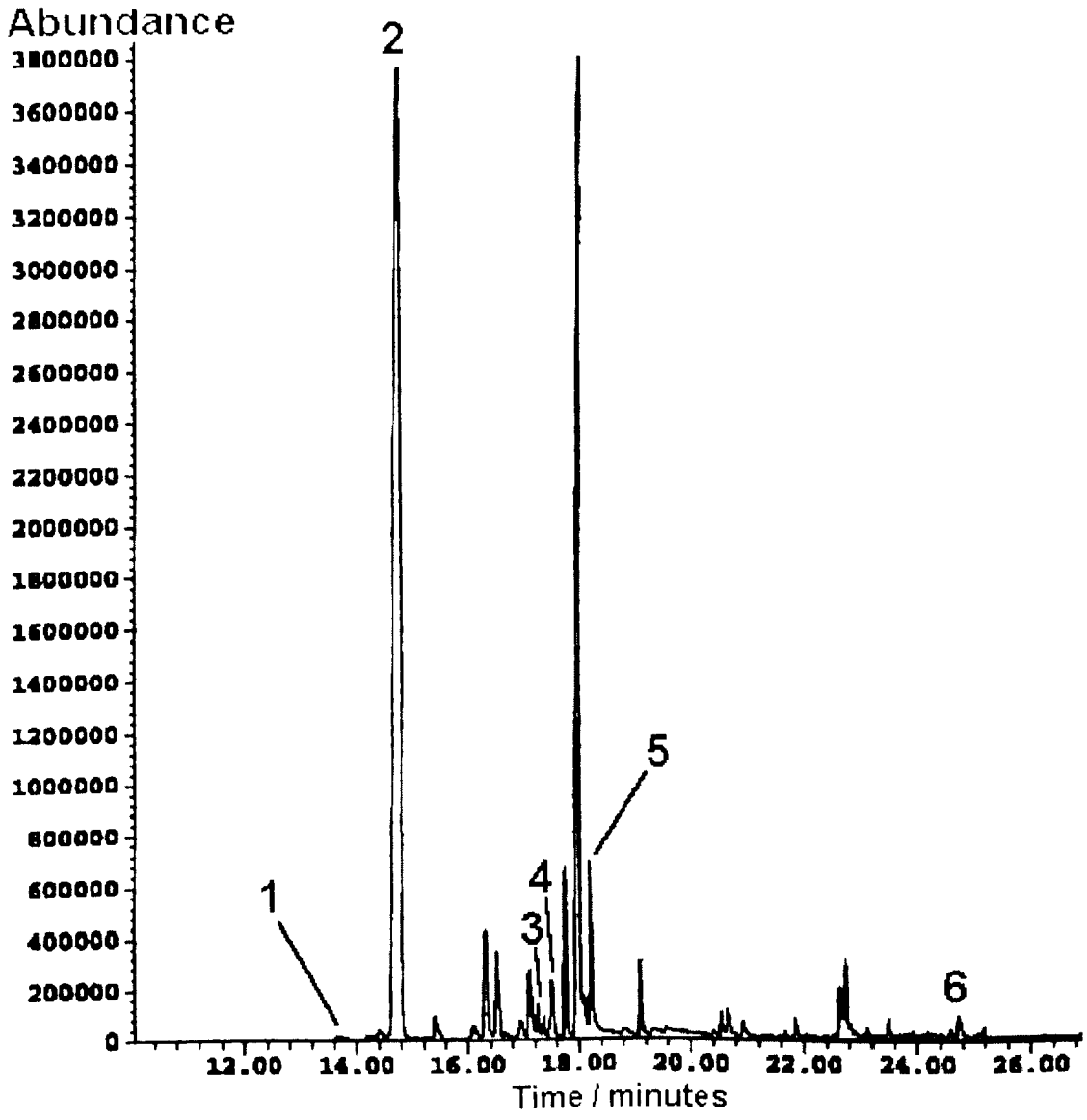


Figure 8.3

A GC-MS chromatogram of the SPME headspace extraction of 'Robinsons orange juice'

1 = β-myrcene, 2 = Limonene, 3 = Sorbic acid, 4 = Decanal

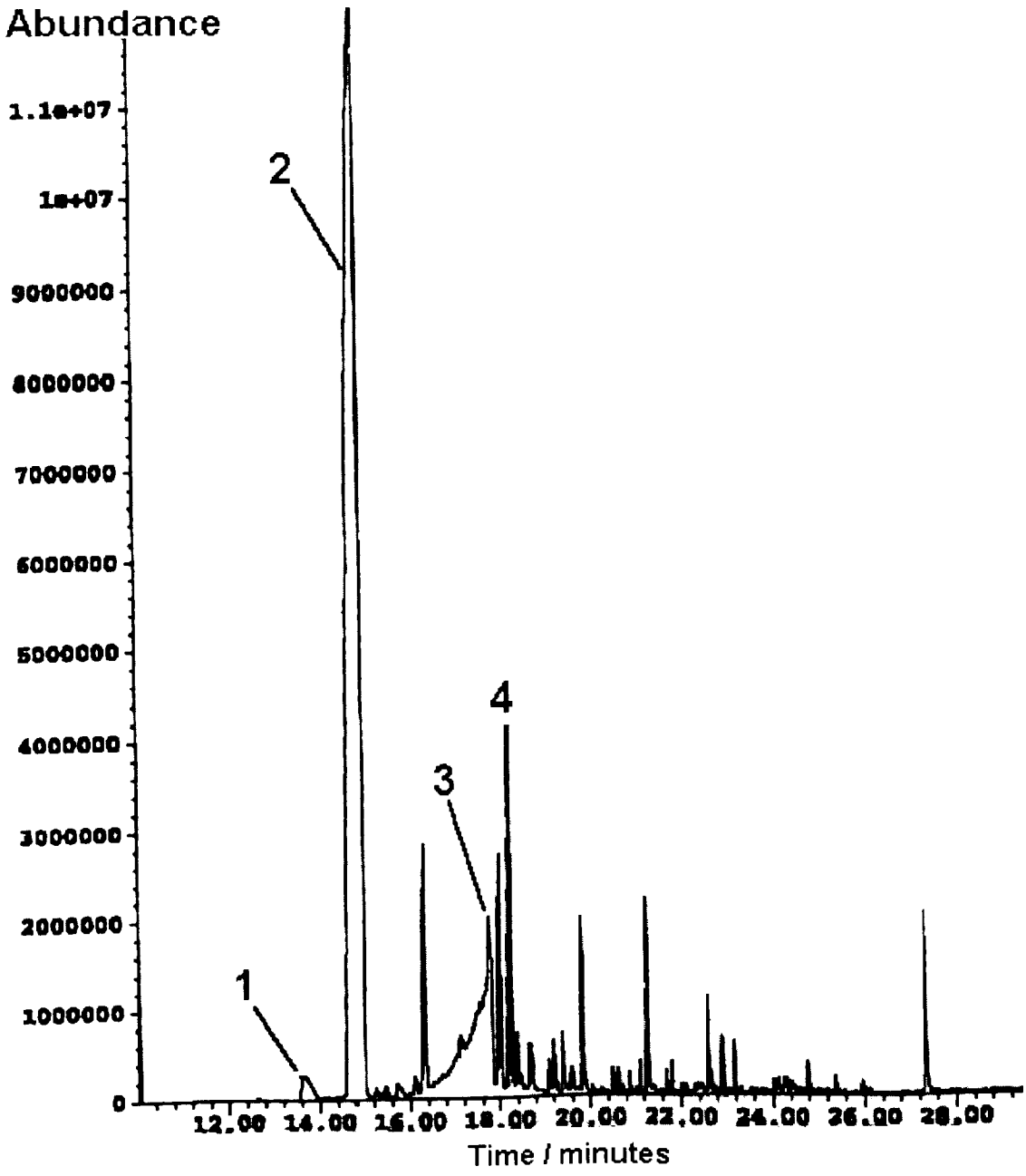


Figure 8.4

A GC-MS chromatogram of the SPME headspace extraction of 'Golden Virginia tobacco'

1 = Nicotine, 2 = Isomers of megastigmatrienone

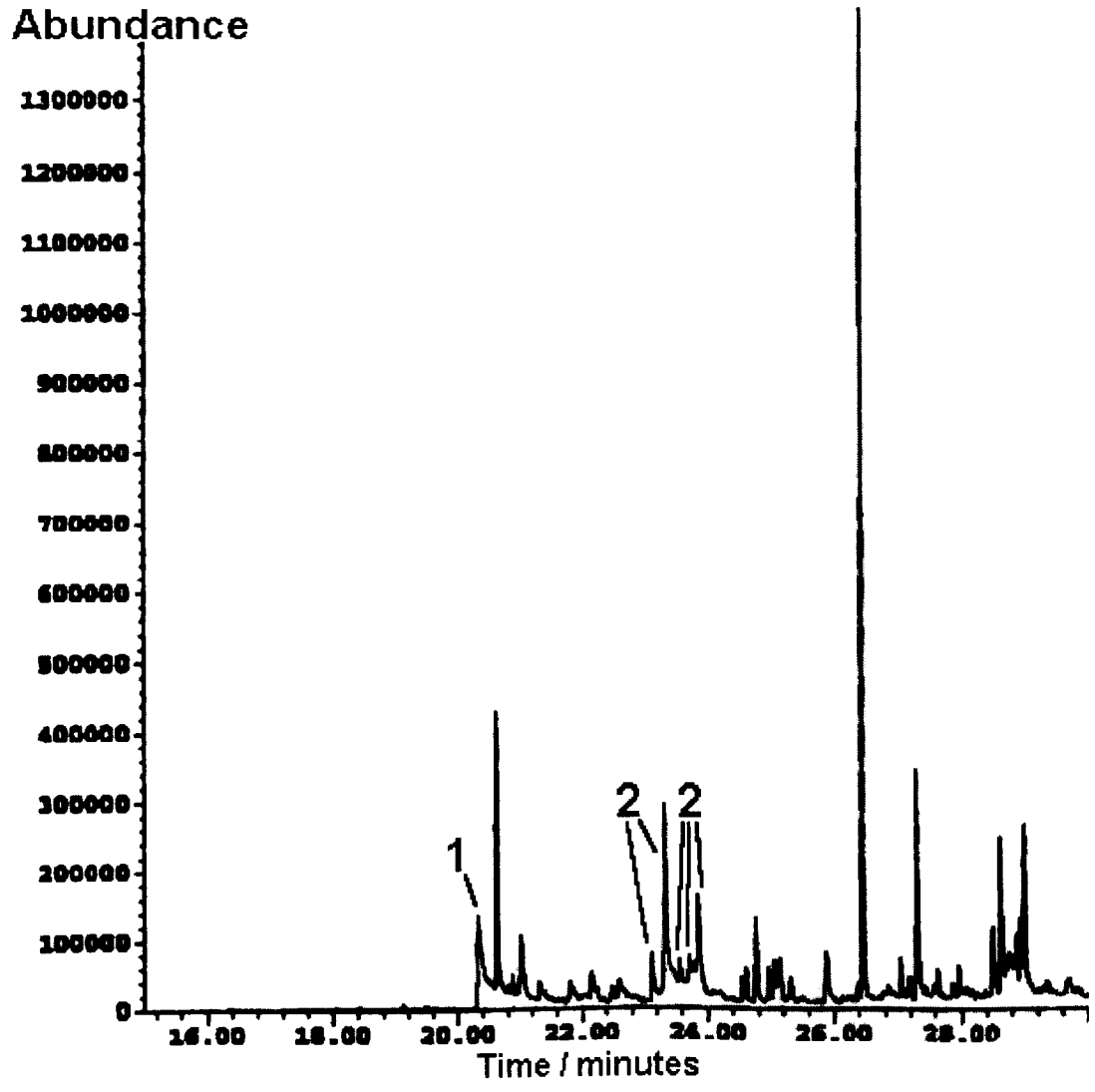


Figure 8.5

A GC-MS chromatogram of the SPME headspace extraction of 'Old Holborn tobacco'

1 = Limonene, 2 = Methylphenols, 3 = Ethylphenols,

4 = Ethylmethylphenols, 5 = Nicotine, 6 = Isomers of megastigmatrienone,

7 = Fluorene, 8 = Phenanthrene, 9 = Anthracene

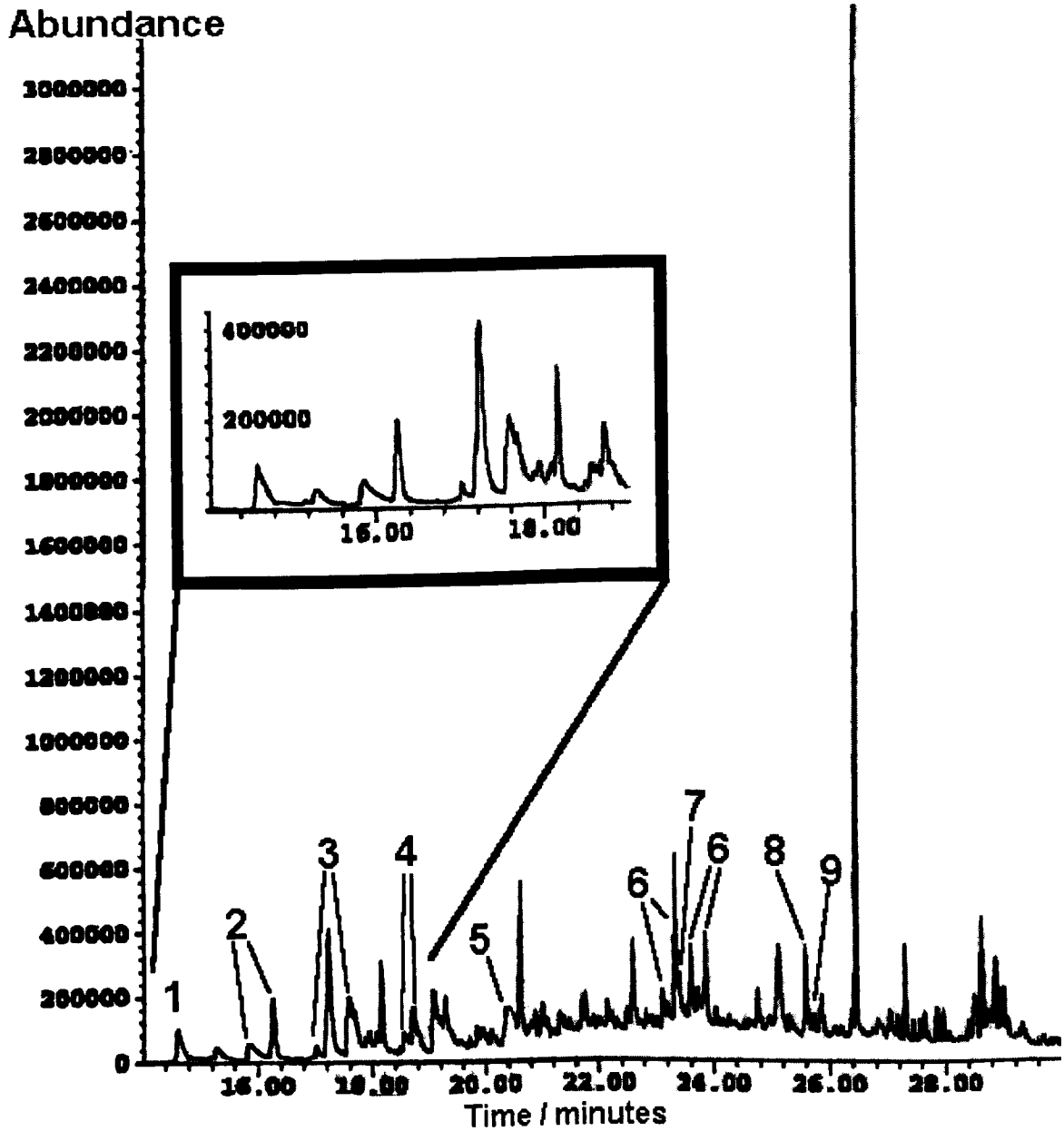


Figure 8.6

A GC-MS chromatogram of the SPME headspace extraction of 'Red Amphora tobacco'

1 = Benzyl alcohol, 2 = Menthol, 3 = Piperonal, 4 = Vanillin, 5 = Ethylvanillin,

6 = Isomers of megastigmatrienone, 7 = Phenanthrene

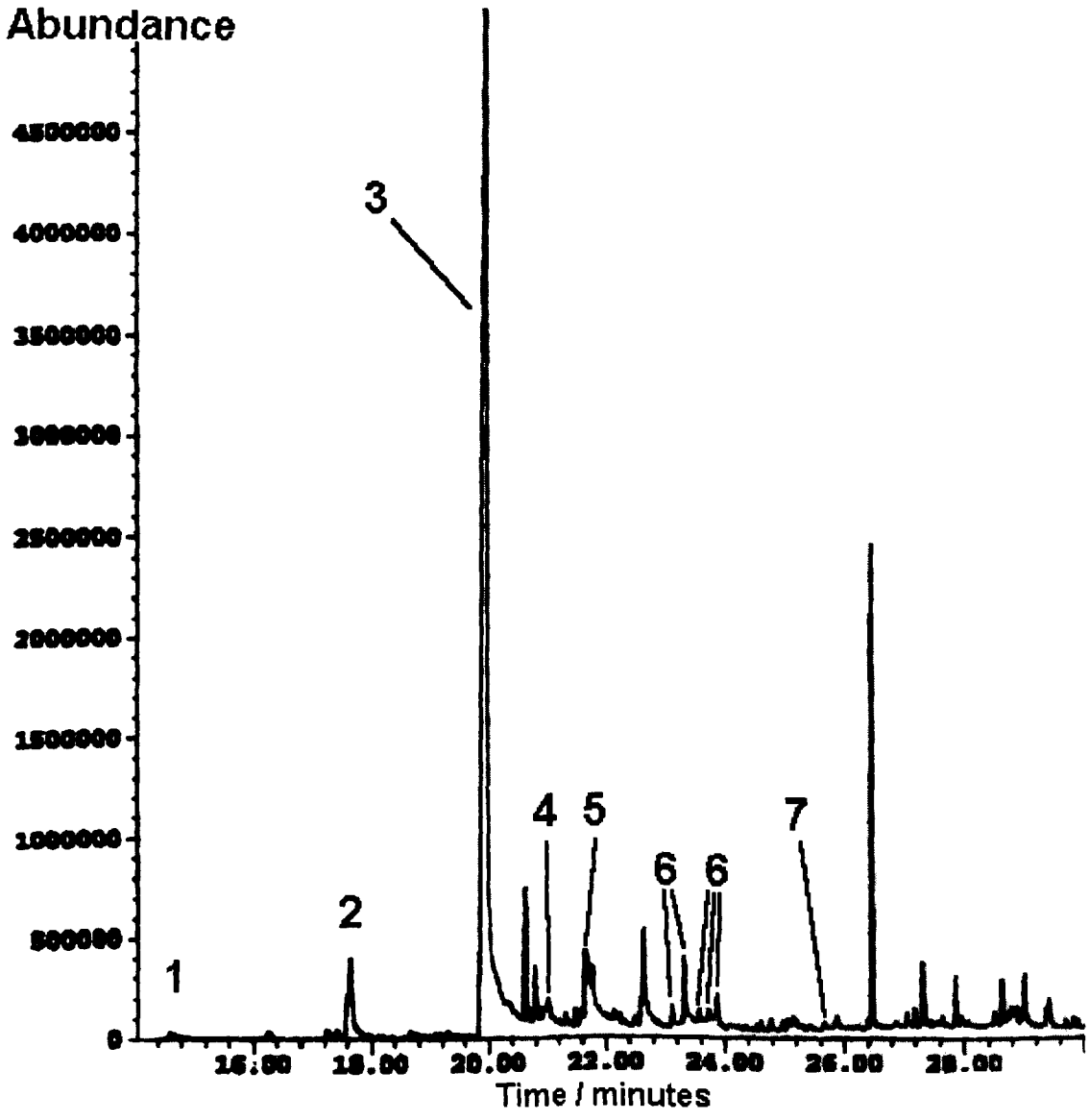


Figure 8.7

A GC-MS chromatogram of the SPME headspace extraction of 'BCR-529 sandy soil'

1 = 1,3,5-trichlorobenzene, 2 = 1,2,4-trichlorobenzene, 3 = 1,2,3-trichlorobenzene,

4 = 1,2,4,5-tetrachlorobenzene, 5 = Pentachlorobenzene, 6 = Isomers of lindane

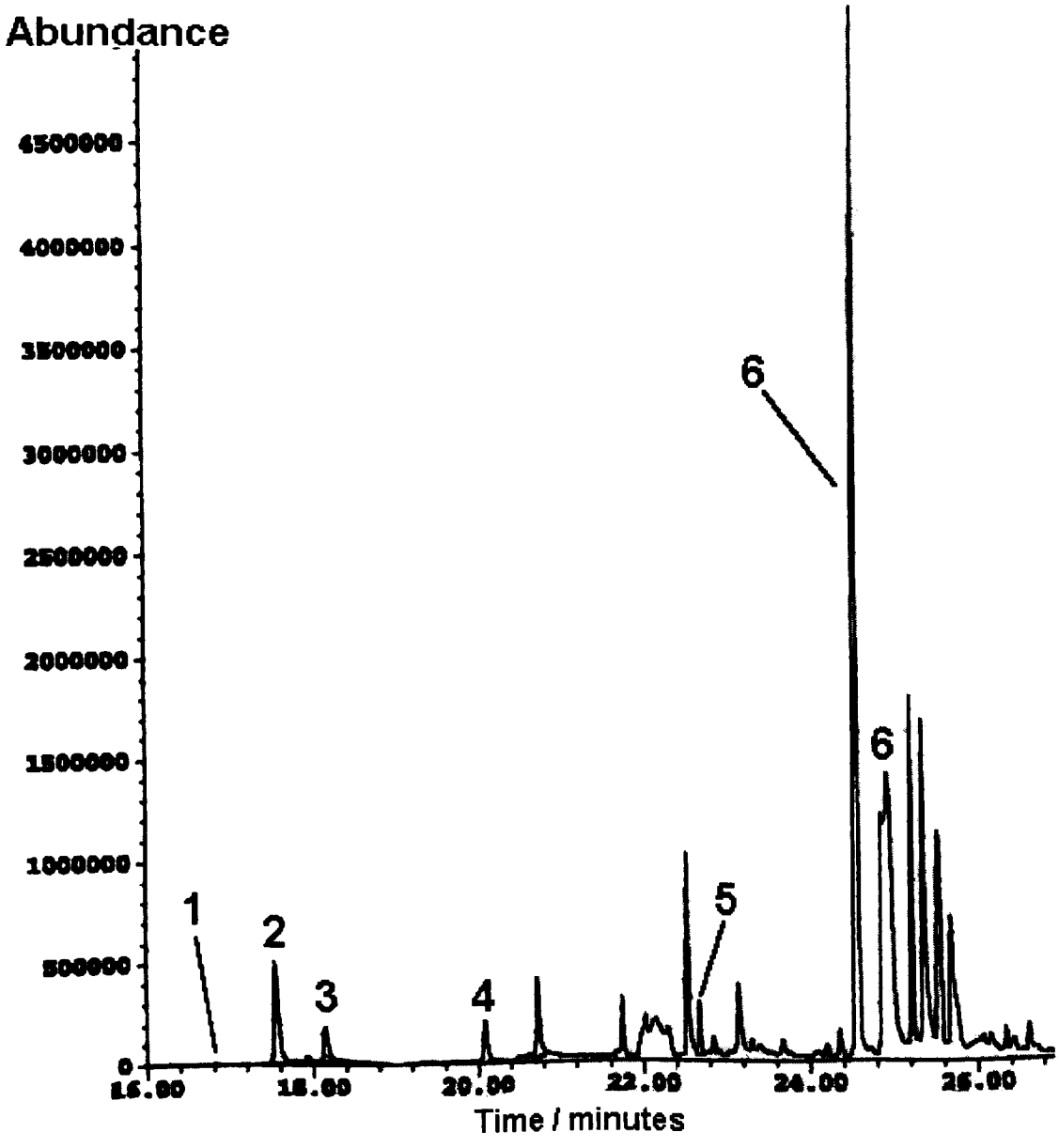


Figure 8.8

A GC-MS chromatogram of the SPME headspace extraction of 'BCR-530 clay soil'

1 = 1,3,5-trichlorobenzene, 2 = 1,2,4-trichlorobenzene, 3 = 1,2,3-trichlorobenzene,

4 = 1,2,4,5-tetrachlorobenzene, 5 = Pentachlorobenzene, 6 = Isomers of lindane

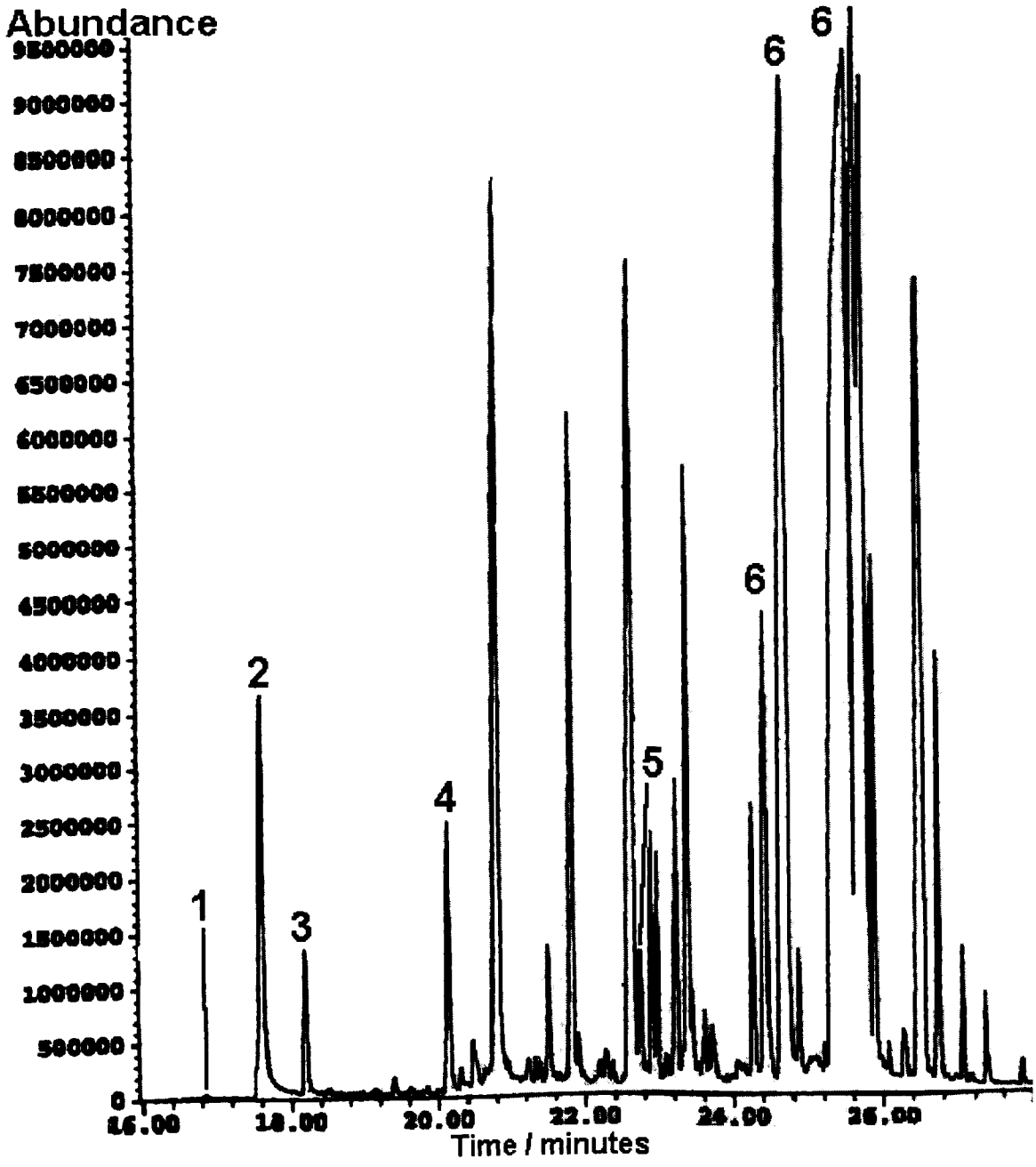


Figure 8.9

A GC-MS chromatogram of the SPME headspace extraction of 'LGC-6138 carbon soil'

1 = Naphthalene, 2 = 2-methylnaphthalene, 3 = 1-methylnaphthalene,

4 = Fluorene, 5 = Phenanthrene, 6 = Anthracene, 7 = Fluoranthene, 8 = Pyrene

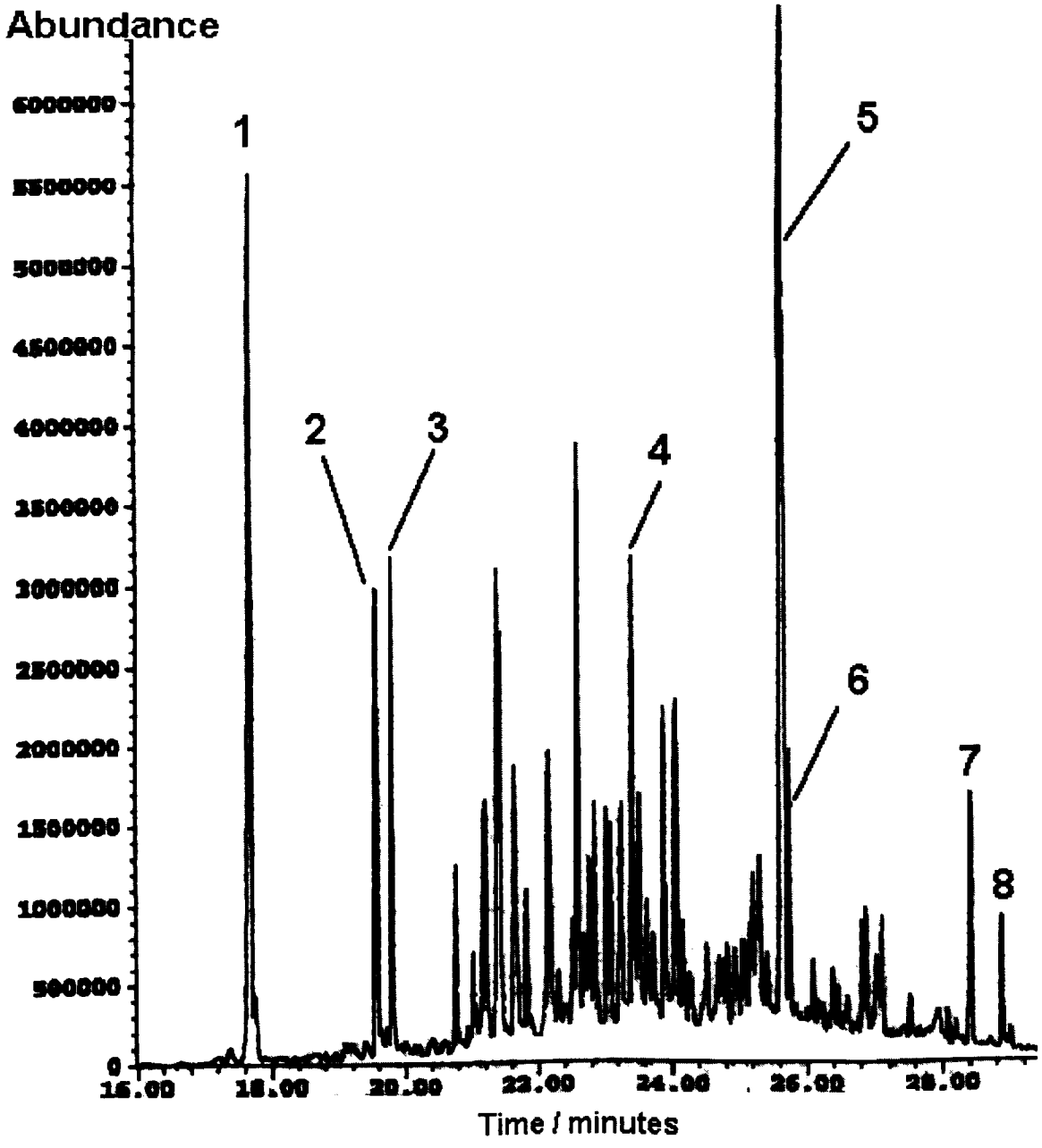


Figure 8.10

A GC-MS chromatogram of the SPME headspace extraction of 'contaminated soil 1'

1 = Naphthalene, 2 = 2-methylnaphthalene, 3 = 1-methylnaphthalene, 4 = Fluorene, 5 = Phenanthrene, 6 = Anthracene, 7 = Fluoranthene, 8 = Pyrene

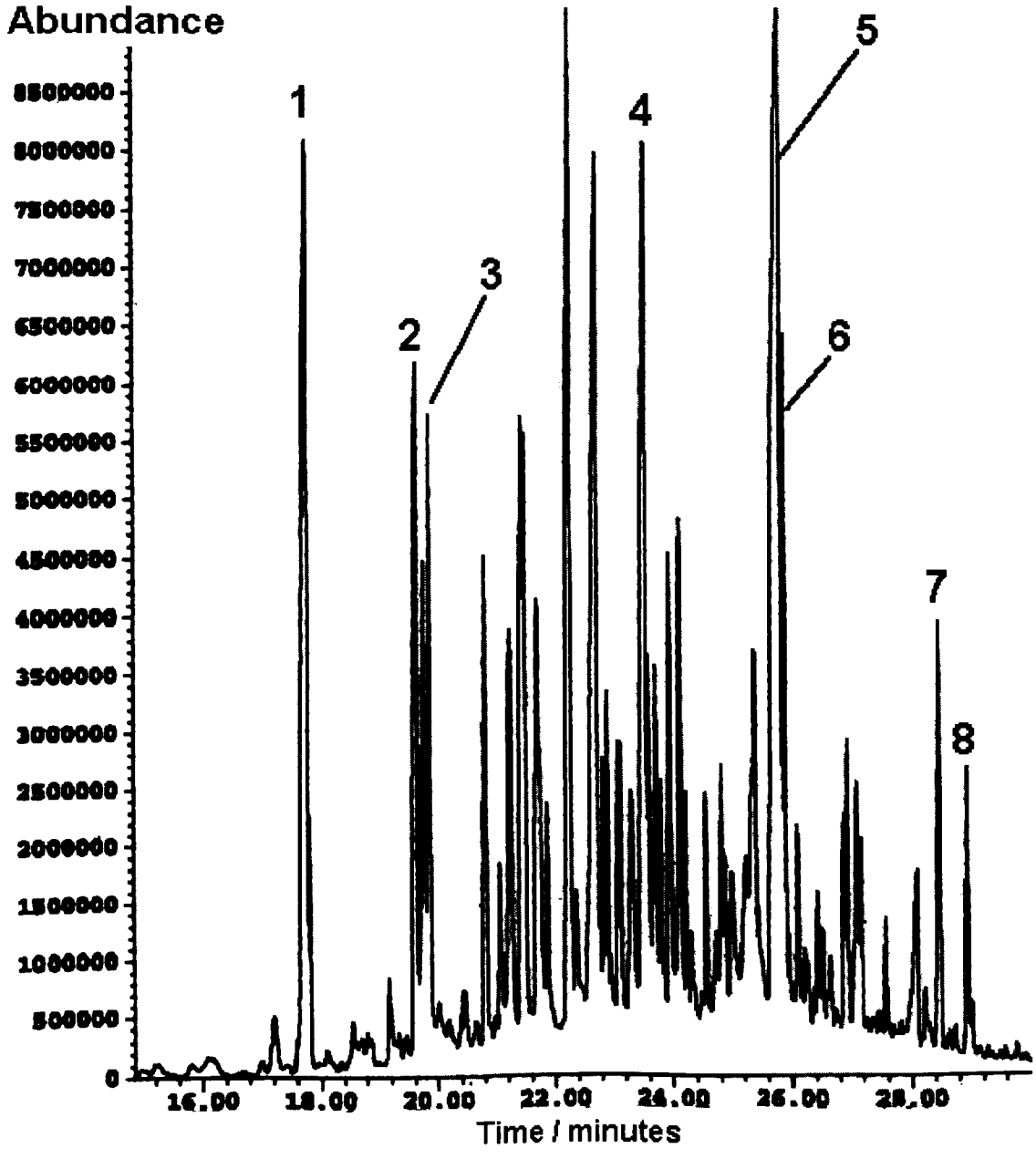


Figure 8.11

A GC-MS chromatogram of the SPME headspace extraction of 'contaminated soil 2'

1 = Naphthalene, 2 = 2-methylnaphthalene, 3 = 1-methylnaphthalene, 4 = Fluorene, 5 = Phenanthrene,

6 = Anthracene, 7 = Fluoranthene, 8 = Pyrene

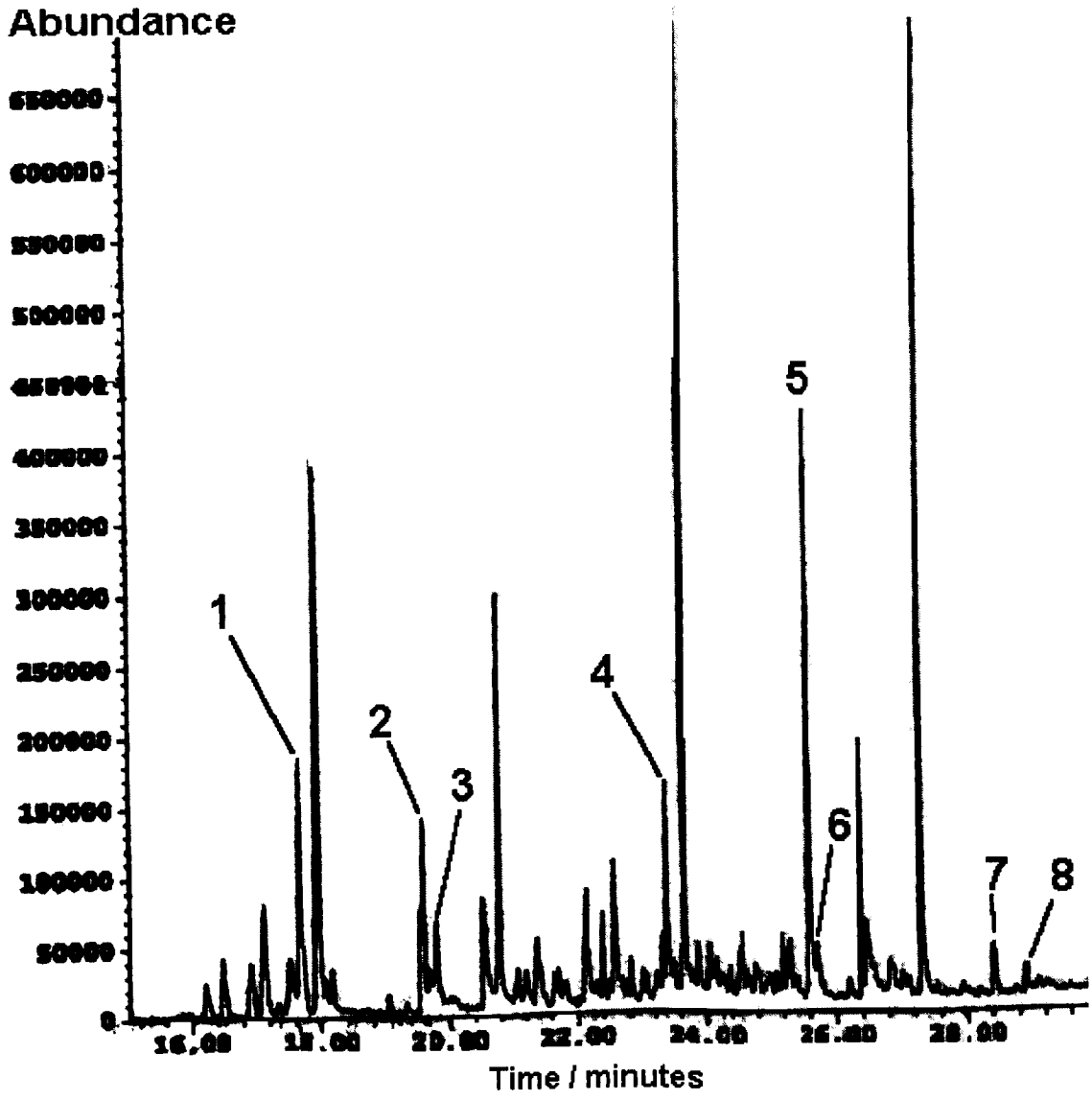


Figure 8.12

A GC-MS chromatogram of the SPME headspace extraction of 'contaminated soil 3'

1 = Naphthalene, 2 = 2-methylnaphthalene, 3 = 1-methylnaphthalene, 4 = Dimethylnaphthalene's, 5 = Trimethylnaphthalenes, 6 = Phenanthrene, 7 = Anthracene, 8 = Fluoranthene, 9 = Pyrene

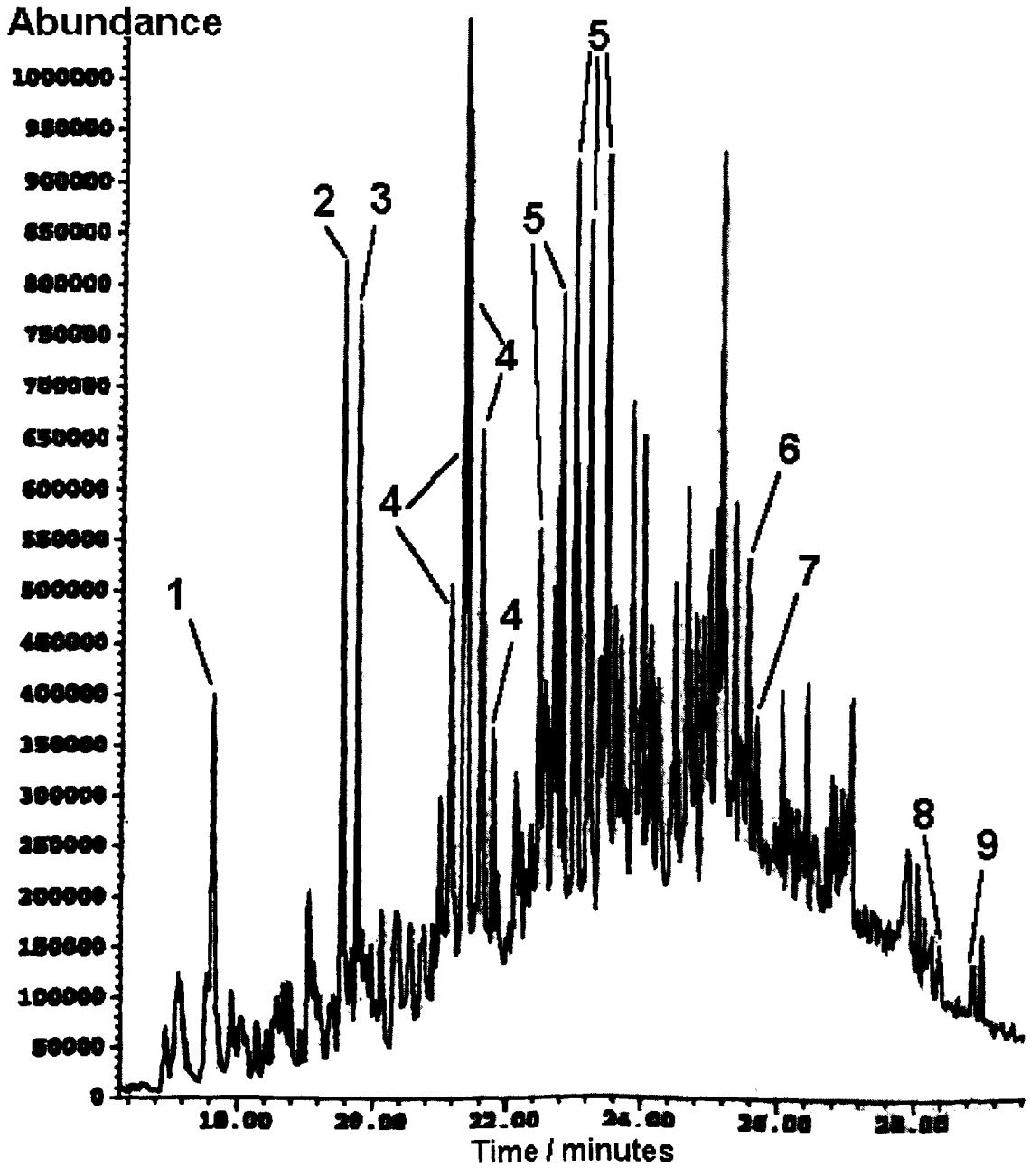


Figure 8.13

A GC-MS chromatogram of the SPME headspace extraction of 'garden soil'

1 = Naphthalene, 2 = 2-methylnaphthalene,

3 = 1-methylnaphthalene, 4 = Phenanthrene, 5 = Anthracene,

6 = Fluoranthene, 7 = Pyrene

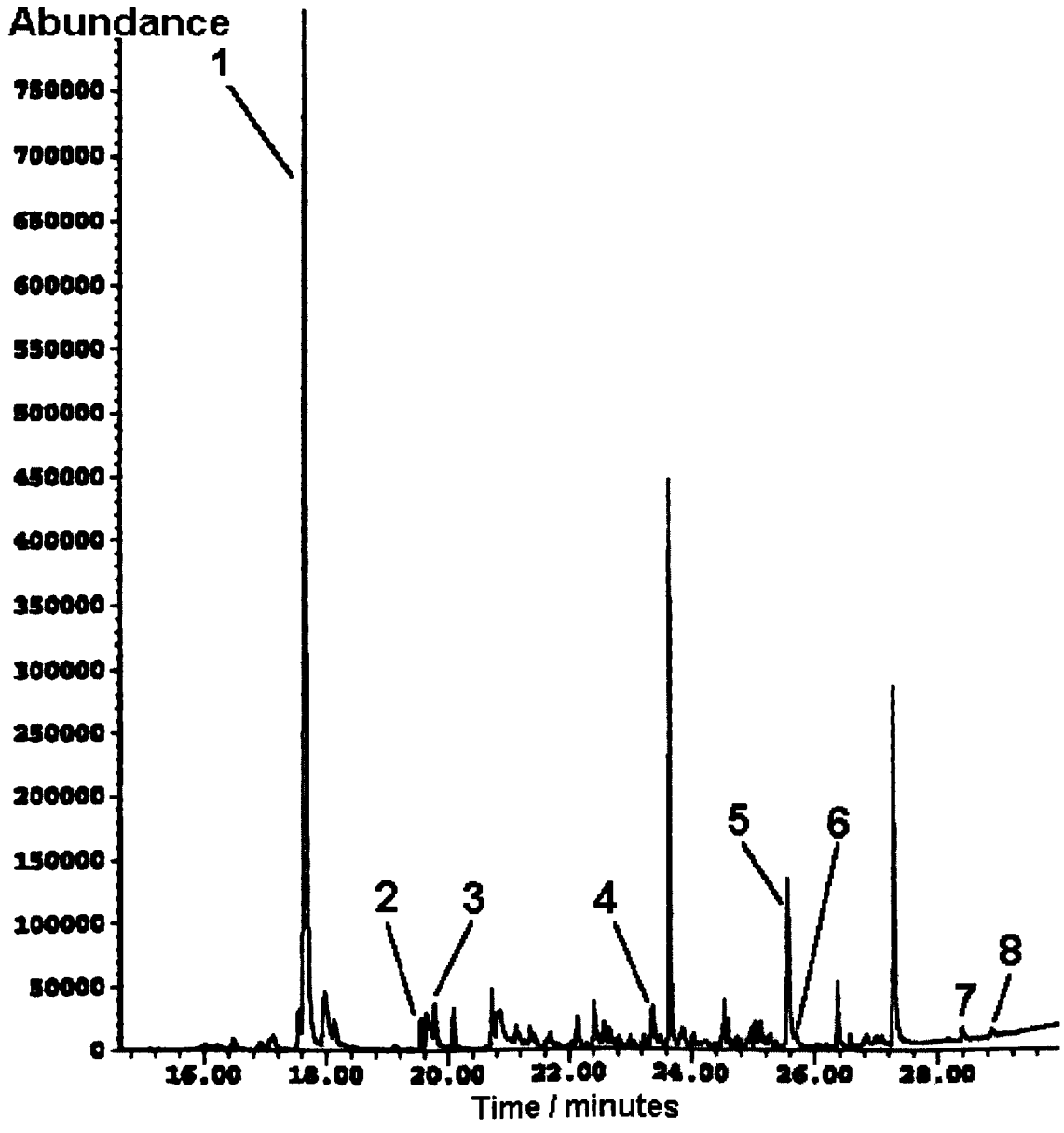
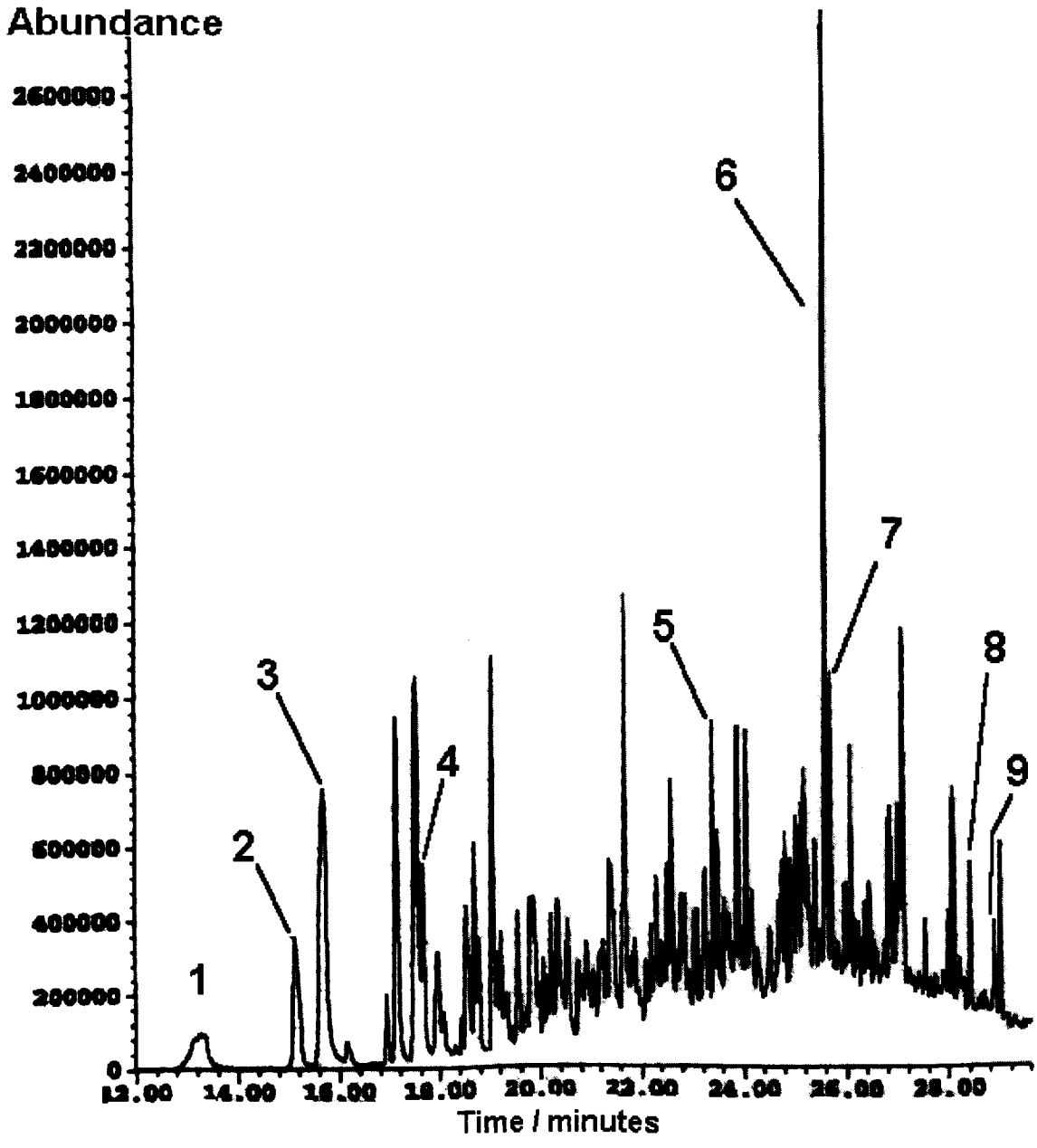


Figure 8.14

A GC-MS chromatogram of the SPME headspace extraction of 'diesel exhaust soot'

1 = Phenol, 2 = Methylphenol, 3 = Ethylphenol,4 = Naphthalene, 5 = Fluorene,

6 = Phenanthrene, 7 = Anthracene, 8 = Fluoranthene, 9 = Pyrene



8.4 Discussion

8.4.1 Soft drinks. *(Figures 8.2-8.3)*

Flavour compounds were identified in the Coca-Cola (figure 8.2) and Robinsons orange juice (figure 8.3). Compounds included limonene and sorbic acid for orange juice and limonene and the fragrance secondary alcohols 'iso borneol' and 'borneol' in Coca-Cola. Decanal was found as a constituent in both soft drinks. Although only qualitative, the presence of limonene would be expected in these matrices since it is a main component in essential oils, such as, mandarin orange oil and sweet fennel oil [1]. The presence of borneol in Coca Cola is more difficult to account for. Borneol is a vasodilator used in the treatment of strokes and is a typical 'drug introducer' in Chinese traditional medicine used to carry other drugs into special organs to enhance efficacy [2]. This may therefore enhance the effect of caffeine in Coca Cola. Borneol has been found in peppermint chocolate bars and is therefore probably a natural constituent occurring in coffee beans [3]. No chlorobenzenes or PAH's were found in these drinks.

8.4.2 Tobaccos. *(Figures 8.4-8.6)*

The tobaccos extracted were 'Old Holborn', 'Golden Virginia' and 'Red Amphora'. The main compound extracted from the pipe tobacco 'Red Amphora' (figure 8.6) was piperonal (a sweet, floral additive). Benzyl alcohol, menthol and small amounts of vanillin and ethyl vanillin (which are also found as a hydrolysis products of lignin in the pulp and paper industry [4]) were also detected. These flavour additives have previously been identified and quantified in tobacco by this same technique [1] and so are

expected to be present. Nicotine was identified in Golden Virginia (figure 8.4) and Old Holborn (figure 8.5), however the peak shape/quality of integration was poor. The huge amount of piperonal in Red Amphora hindered identification of nicotine. The use of selected ion monitoring could greatly enhance the detection and identification of nicotine.

The Old Holborn extract yielded a host of early eluting methyl, ethyl and ethylmethyl phenols. As these occur in the early stages of the chromatogram they are obviously relatively volatile compounds which undoubtedly contribute to the strong pungent smell of Old Holborn. Golden Virginia has very little smell in coincidence with the lack of any volatiles in the chromatography. However, Red Amphora has a pleasant fruity smell and there are no significant early eluting compounds as in the case of Old Holborn. The scent of Red Amphora is therefore attributed to the large concentration of semi-volatile piperonal and to a lesser extent menthol and the vanillin compounds.

Isomers of megastigmatrienone were found to be present in all three tobaccos whilst the PAH's fluorene, phenanthrene and anthracene specifically occurred in Old Holborn. A strong scent is not solely attributed to volatile compounds although these compounds will be in high concentrations above a sample. The nature and strength of the scent we detect also depends on the electronic structure of a molecule which will induce a response by interacting with scent receptors in the nose. For example, megastigmatrienone is a semi-volatile (inferred from its retention in GC) C_{13} degradation product of the carotenoids in tobacco and is said to be the most important flavour and fragrance component in tobacco, possessing a strong plum-like odour [5]. However, the lack of scent of Golden Virginia which also possesses megastigmatrienone isomers doesn't account for this. Since the levels of megastigmatrienone in all the tobaccos are

similar, it can be concluded that this concentration is not enough to cause a scent response.

Previous work on analysing tobacco volatiles from the headspace have been complicated and demanded skill [6]. For example Austin et al. [7] collected the volatiles from 3.5 kg of cut tobacco with 75 g of active carbon over 2 weeks whilst Ito et al. [8] developed a more rapid method with both Tenax GC trap (a porous polymer) and activated carbon followed by GC analysis. However both these methods require lengthy purification stages to regenerate the sorbents and large sample mass and require a high degree of manual skill. In comparison, the regeneration of the SPME fibre is accomplished in a matter of minutes after analyte desorption in the injection port.

8.4.3 Reference materials (RM's). *(Figures 8.7-8.9)*

Only information concerning several chlorophenols, 1,2,3-trichlorobenzene and pentachlorobenzene was available for the sandy BCR soil. However, trichlorobenzenes, tetrachlorobenzene and pentachlorobenzene were identified as being present in both the sand (figure 8.7) and clay (figure 8.8) BCR reference soils as well as a substantial amount of the lindane isomers (hexachlorocyclohexane). All the PAH's chosen were also identified in the LGC reference material (figure 8.9). One important characteristic in the analysis of the extractions was that no phenols were extracted from the BCR reference soils and that no sulphur was extracted from the LGC soil. Sulphur is present in the LGC soil at a concentration of c.a. 45900 mg/kg compared with the presence of PAH's (between 15.3-118 mg/kg). This extraction selectivity highlights the great advantage of SPME over other procedures which require lengthy sample clean-up steps.

8.4.4 Contaminated soils and garden soil. *(Figures 8.10-8.13)*

The three industrially contaminated soils all contained the PAH's previously identified as present in the LGC reference material. Contaminated soil No. 3 also contained many dimethyl and trimethyl naphthalenes. In comparison to the heavily contaminated soils (figures 8.10-8.12), garden soil (figure 8.13) showed much lower amounts of the PAH's, the most prevalent being naphthalene. Fluorene was not present in contaminated soil No. 3 or garden soil. None of these samples showed the presence of chlorobenzenes.

8.4.5 Diesel exhaust soot *(Figure 8.14)*

This matrix contained phenol, methylphenol and ethylphenol along with all the PAH's found in the LGC reference material.

8.5 Summary and conclusions

The SPME chromatograms presented in figures 8.4-8.6 can be viewed as a fingerprint of tobacco aroma which is one of the most important characteristics of estimating tobacco quality. Over 4000 substances have been determined in smoke condensate [9] including water, nicotine, alkaloids [10-11], PAH's, N-heterocycles, nitrosamines [12], hydrogen cyanide, hydrogen sulphide [13], pesticides, organic acids, keto compounds, phenols, metals [14], tar, sugar and polyphenols [15]. Many methods therefore exist to monitor these different chemical substances some of which can be complicated and time consuming. However increasing analytical demands are putting

pressure on conventional time consuming analytical methods, such as, Soxhlet. The separation method used was specifically for the chlorobenzenes and PAH's under analysis. However, this separation and extraction method can be (and has been) modified to extract and analyse most of the above mentioned compounds [16-21]. SPME could be used for rapid automated screening and be incorporated into tobacco processing lines to provide information on the quality characteristics of the raw material, semi-finished and finished products, and to monitor the physiologically active components of smoke. SPME would therefore have the advantage over the many different methods traditionally used, as a universal method which is fast and automatable with possibilities of quantitation.

As with tobacco, the flavour quality and ripeness of soft drinks and fruits can be monitored by screening the aroma producing compounds. This provides the basis for quality control of fresh and processed products. SPME overcomes traditional sampling methodologies in aroma research such as purge and trap, which are also more expensive and time consuming. SPME has been used to further enhance sensitivity in the monitoring of flavour and fragrance compounds in many matrices. These include fruit beverages [21-22], peppermint cookie bars, ginger/lemon and lavender oils and white pine leaves [3]. Amongst the common chemicals determined in these matrices were limonene, β-myrcene, α-pinene and camphene, whilst menthol and borneol were specific to cookie bars and lavender oil respectively. Flavours have also been extracted from chewing gum and crisps by microwave extraction with water followed by further extraction by SPME [23].

Of the matrices analysed by this method, all the soils and diesel exhaust soot were chosen for further quantitative analysis of chlorobenzenes and PAH's by SPME (chapter

10). The ability to accurately quantify these compounds in solid matrices by SPME is of paramount importance since the present accepted methods of analysis are time consuming and are becoming environmentally and economically unfeasible. The aim of further studies was to employ SPME with GC-MS / ECD to develop a single solvent free method able to rapidly extract and quantify these compounds in liquid (chapter 9.0) and solid (chapter 10.0) matrices. It is hoped that the use of method optimisation studies will allow an understanding of the factors that govern the mechanisms present in partitioning. This will help to gain a wider understanding of the implications that the results from these investigations will suggest.

8.6 References

1. T. J. Clark and J.E. Bunch, *J. Agric. Food Chem.*, **45** (1997) 844.
2. M. Yang, S.G. Lin ,T. Wu, and T.F. Chen, *Asia Pacific J. Pharmacol.*, **4** (1989) 1.
3. SPME Supelco Bulletin **869** (1995) 1.
4. J.M. Davidson and J.R. McDougal, *J. Environ. Qual.*, **2** (1973) 428.
5. P. Weyerstahl and K. Licha, *Leibigs Ann. Recuiel.*, **1** (1997) 1919.
6. T. Sakaki, K. Niino, H. Sakuma and S. Sugawara, *Agric. Biol. Chem.*, **48** (1984) 3121.
7. D.J. Austin, J. Roeraade, B. Kimlandd, A.J. Austin and C.R. Enzell, Beitr.Tabakforsch, **7** (1973).
8. N. Ito, T. Etoh, H. Hagiwara and M. Kato, *J. Chem. Soc. Perkin Trans.*, **10** (1997) 1571.
9. R.E. Fresenius, *J. Anal. Applied Pyrolysis*, **8** (1985) 561.

10. R.F. Severson, K.L. McDuffie and R.F. Arrendale, *J. Chromatogr.*, **211** (1981) 111.

11. F. Manceau, M.A. Fliniaux and A.J. Dudreuil, *Phytochem. Anal.*, **3** (1992) 65.

12. K.D.Bronnemann, ACS symposium series, chap 45, **553** (1994) 369.

13. E. Makleit-Szabo, *Acta Alimentaria.*, **10** (1981) 337.

14. G. Schneider and V. Krivan, *Intern. J. Environ. Anal. Chem.*, **53** (1993) 87.

15. M. Varadi, W. Hruschka and K.H. Norris, *Acta Alimentaria,* **21** (1992) 95.

16. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 160.

17. I.J. Barnabas, J.R. Dean, I.A. Fowlis and S.P. Owen, *J. Chromatogr. A,* **705** (1995) 305.

18. Z. Zhang and J. Pawliszyn, *Anal. Chem.*, **65** (1993) 1843.

19. S.S. Johansen and J. Pawliszyn, *J.High Resolut. Chromatogr.*, **19** (1996) 627.

20. L. Moens, T.D. Smaele and R. Dams, *Anal. Chem.*, **69** (1997) 1604.

21. Z. Penton, SPME Varian Application Note **11**, (1996).

22. J. Song, B.D. Gardner, J.F.Holland and R.M. Beaudry, *J. Agric. Food Chem.*, **45** (1997) 1801.

23. Y. Wang, M. Bonilla and H.M. McNair, *J. High Resolut. Chromatogr.*, **20** (1997) 213.

Chapter 9.0

Quantitative aspects of SPME: Water extraction

9.1 Introduction

The method developed to separate chlorobenzenes and PAH's given in the previous chapter was applied in this study. Chlorobenzenes were analysed by GC with electron capture detection (ECD) and PAH's were analysed by GC with mass spectral detection (MS). Firstly, a fully automated SPME method was optimised for the extraction and quantification of chlorobenzenes directly from water. Secondly, a manual method was evaluated for the extraction and quantitation of PAH's from water.

Partition coefficients were calculated for direct extraction at equilibrium of chlorobenzenes with both fibres (K_{fs}^{PA} and K_{fs}^{PDMS}) and by direct and headspace extraction at 30 mins for PAH's with both fibres (K_{fs}^{PA}, K_{fh}^{PA} and K_{fs}^{PDMS}, K_{fh}^{PDMS}). The effect of temperature and agitation on the extraction and equilibration time was evaluated for the extraction of PAH's. The implications of the results of these studies are discussed. Correlations were found between partition coefficients and various physical constants of chlorobenzenes and PAH's. The correlations between K_{fs}^{PDMS} and Kow for chlorobenzenes and PAH's were combined to develop a model to predict partitioning (at equilibrium) of analytes from different chemical groups based on their literature Kow values. The predicted values were then compared with experimental values found in the literature for SPME partitioning.

9.2 Experimental

9.2.1. Chlorobenzenes

Primarily a standard series of concentrations were prepared in hexane for direct injection. 1 μl of each concentration was analysed and calibration curves generated from the results. Due to the insoluble nature of chlorobenzenes in water c.a. 4000 μmoles/dm^3 stock solution was prepared in acetone. Water standards were prepared by serial dilution into HPLC grade water prior to extraction. Photoreduction (reductive dechlorination) of chlorobenzenes can occur in organic solvents such as acetone which act to sensitise the compounds under certain UV wavelengths [1]. Since standard preparation required small amounts of acetone, standards were stored in amber vials at low temperatures to minimise this effect.

All automated SPME work was static as dictated by the equipment used. The SPME autosampler was housed above the injection port of the GC. An SPME device replaced the usual direct injector syringe in the set-up, and the circular carousel below could hold over 50 samples. Extractions were controlled by the software of the GC which allowed the programming of individual parameters for each sample. 1 ml of each sample was pipetted into 2 ml autosampler vials which were then sealed either with a screw top or crimp top and placed in the carousel of the autosampler. Each extraction was carried out with a fresh solution.

The parameters studied for optimisation were equilibration sorption time, desorption time and fibre coating type. Repeatability of the extractions for sorption

equilibration were found, and optimised parameters were chosen which gave high sensitivity in the minimum amount of time. These conditions were employed to find the limits of detection of each chlorobenzene. The linearity of the concentration range was studied and sensitivity was then compared with direct injection detection limits. To monitor the repeatability of the method, repeated extractions were carried out for concentrations within the working linear range and compared with the repeatability of direct injections.

The performance of the 85 μm polyacrylate (PA) and 100 μm polydimethylsiloxane (PDMS) fibres was compared. Each fibre was exposed to a 1 ml solution of the same concentration (c.a. 55 nmoles/dm^3) for differing lengths of time to asses analyte equilibration times. All sorption curves were carried out over times ranging from 0.01-120 mins and the nmoles sorbed by the fibre were calculated from direct injection of external calibration solutions. The partition coefficients were calculated at 120 mins which was at c.a the equilibration time. The linear concentration range, limits of detection (method sensitivity) and repeatability of the two fibres were estimated. The extraction kinetics were monitored using polyacrylate (PA) and polydimethylsiloxane (PDMS) coated fibres. The injection port temperature was 280 °C when using PA fibre and lowered to 250 °C when using the PDMS fibre due to the lower thermal stability of the PDMS coating.

The fibre coating is used to concentrate analytes from a volume 1000 times larger than that of direct injection. Therefore the amount deposited on the column is higher by SPME than for the same sample concentration directly injected. This results in a higher sensitivity than direct injection, for example:

A 1 μl direct injection of 41.6 nmoles/dm^3 PCB resulted in an area = 22721

A 20 min analysis by SPME of 1 ml 35.2 nmoles/dm^3 PCB resulted in an area = 739551

As the split ratio is the same for direct injection as SPME, so the detector response generated by both are equally relative to the concentration. This increased sensitivity of SPME compared with direct injection results in lower limits of detection allowing lower concentrations to be analysed.

The volumes for each fibre were calculated by subtracting the volume of the silica support from the total volume of the silica + coating. To allow direct comparison of the partition coefficients of the fibres, the volume of each fibre is accounted for in the results (eqn. 7.5). Optimum desorption time was found by exposing fibres to solutions of the same concentration (c.a. 55 nmoles/dm^3) for a set time (20 mins), before desorption for progressively longer times until blank desorptions carried out between each desorption showed no carryover. 1,2,4-trichlorobenzene is chosen to illustrate the results from these experiments.

Log Kow and Henry's constants are often used to explain the results of partitioning in the literature [2-6]. Therefore, correlations by linear regression (R^2) were found between Kfs^{PA} or Kfs^{PDMS} of the chlorobenzenes at equilibrium and their respective Henry's constants and log Kow's. Literature values for Henry's constants and log Kow, of the chlorobenzenes are given in table 9.1.

Table 9.1

Characteristic properties of chlorobenzenes

	log Kow [ref]	Henry's constant $Pa.m^3.mol^{-3}$ [2]
1,3,5-trichlorobenzene	4.19 [3]	193
1,2,4-trichlorobenzene	4.27 [4]	122
1,2,3-trichlorobenzene	4.27 [4]	90
1,2,4,5-tetrachlorobenzene	4.6 [5]	101
Pentachlorobenzene	5.2 [6]	71.9

The condition of the polyacrylate fibre was monitored over the time of usage. Fibres at 3 stages of use were photographed under magnification by scanning electron microscopy (SEM). These fibres were from use in experiments involving the extraction of chlorobenzenes from HPLC grade water.

9.2.2 PAH's

Due to the limitations posed by the automated equipment used with chlorobenzenes, two manual methods of extraction were investigated with PAH's. This allowed the flexibility to study the effects of temperature and agitation on sensitivity. These parameters have been found to be beneficial to the extraction process in different ways. An increase in temperature has been shown to linearly increase Henry's constants [7], whilst agitation techniques have been shown to speed up the attainment of equilibrium [8]. The effect of temperature was investigated on PAH extraction in closed systems. The effect of temperature and agitation on the extraction of PAH's was investigated using solutions in open vials. Like chlorobenzenes, PAH's have a low

solubility in water, and aqueous solutions had to be prepared by serial dilution of a stock solution prepared in acetone.

9.2.2.1 Closed systems

Time/sorption profiles were carried out on 1 ml (c.a. 2 μmoles/dm^3) aqueous solutions in sealed 2 ml vials between 1-30 mins. These solutions were analysed by direct and headspace extraction at 23 °C (room temperature) and at 85 °C. Vials were heated by lowering them into a water bath at a pre-set thermostatted temperature which was monitored using a thermometer. The samples were allowed to equilibrate for 1 minute prior to exposing the fibre.

The water bath apparatus used in these experiments was shown in figure 8.1 chapter 8.0. Samples which were not heated were also lowered into the water bath at room temperature to minimise the effects of fluctuations in laboratory temperatures. The ambient temperature of water was measured as 23 °C.

The direct analysis at 85 °C was only carried out at 30 mins to compare the chromatography and partitioning coefficients with the other modes of extraction. Both the 100 μm PDMS and the 85 μm PA fibres were compared under these conditions. Repeats were carried out at chosen times under the different conditions to assess repeatability of the manual technique.

As in the case with chlorobenzenes, direct injections of external standards were used to quantify the levels extracted by SPME. Standard calibration curves were

generated under the same conditions optimised for the extraction of chlorobenzenes in order to assess detection limits. Correlations were made between the partition coefficients of the PAH's calculated at 30 mins and their respective boiling points, solubilities, vapour pressures, Henry's constants and log Kow's, in order to understand which factors may contribute to the extraction process. These properties are given below in table 9.2.

Table 9.2

Characteristic properties of PAH's

	log Kow	Henry's constant (H) $Pa.m^3.mol^{-1}$	Solubility (Cs) $mol.m^{-3}$	Vapour pressure (Ps) Pa	Boiling point (BP) °C
Naphthalene	3.36	44.6	0.242	10.4	217.7
1-methylnaphthalene	3.95 [9]	24.3	0.197	8.84	242
Fluorene	4.47	9.75	0.0114	0.09	298
Phenanthrene	4.57	3.61	0.00617	0.02	336
Anthracene	4.54	7.66	0.00025	0.001	340
Fluoranthene	5.22	0.65	0.00129	0.00123	384
Pyrene	5.18	1.21	0.00065	0.0006	404
Reference	[9-10]	[2]	[2]	[2]	[Aldrich]

9.2.2.2 Open systems

Based on the enhanced extraction characteristics for PAH's found with the PDMS fibre, it was used in further experiments where magnetic stirring was employed to monitor the effects of agitation on extraction at 23 °C and 85 °C. These extractions were carried out in open systems. 10 ml solutions of PAH's were extracted from 20 ml vials. The concentration of the solutions used in these experiments were also c.a. 2 μmoles/dm^3.

However the higher volume used dictated a higher total mass of analytes in the system. This higher mass was used because it was believed that using a lower mass may result in exhaustive extraction under conditions favourable for equilibrium (i.e. stirring).

The PAH solutions were all analysed directly, 'with' and 'without' stirring, at 23 °C and 85 °C between 1-60 mins. Heating was carried out using the water bath apparatus. All vials were lowered into the water to the same distance from the hot plate magnetic stirrer. A distance was chosen so that stirring could be accomplished without the vial sitting on the bottom of the water bath thus avoiding a more direct contact with the heat source. The stirrer speed was kept constant by monitoring the depth of the vortex produced. A straight stir bar which was close to the diameter of the vial was found to work best.

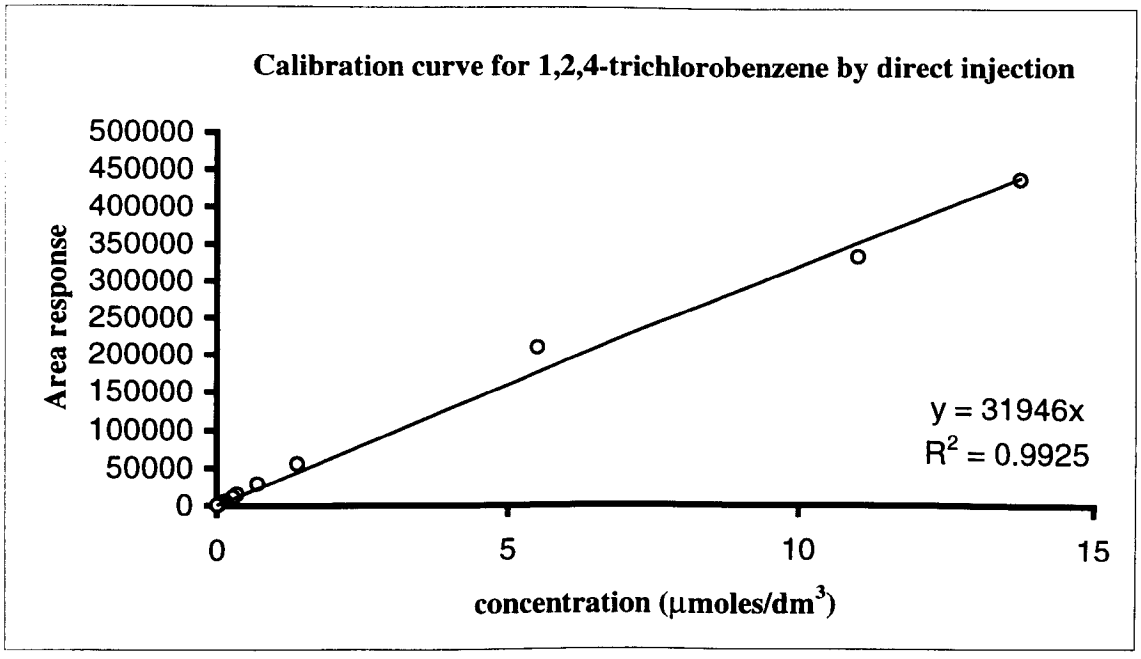
9.3 Results and discussion

9.3.1 Chlorobenzenes

9.3.1.1 Direct injection

Calibration curves by direct injection of 'concentration versus peak area' plotted for each analyte showed linearity between c.a. 0.01-14 μmoles/dm^3. For example a curve for 1,2,4-trichlorobenzene is shown in figure 9.1.

Figure 9.1



Detection was limited to concentrations above 0.01 μmoles/dm^3. At higher concentrations than 14 μmoles/dm^3 the line began to plateau. Repeatability of injection (n = 15) gave RSD's between 8.5-11.1 %.

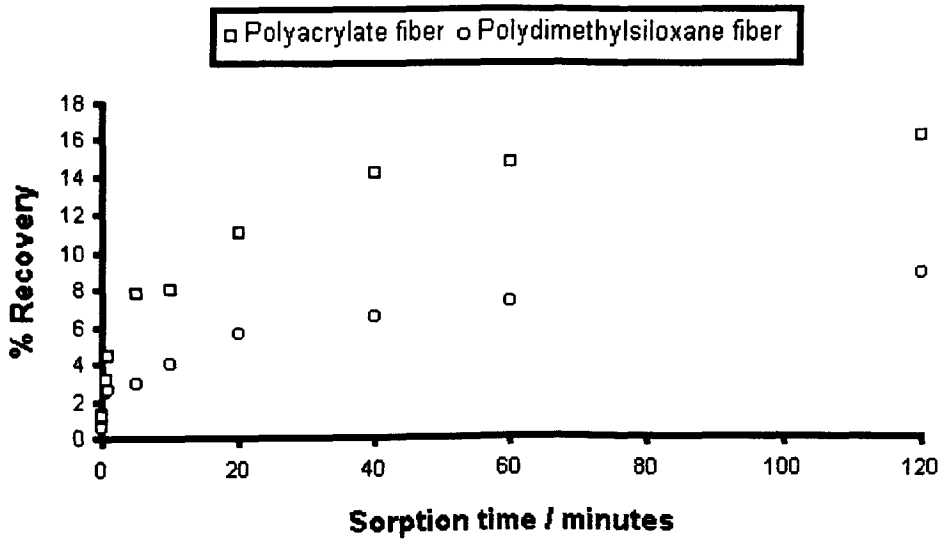
9.3.1.2 Sorption equilibration

A comparison of extraction recoveries versus time for the extraction of 1,2,4-trichlorobenzene using both PA and PDMS fibres is shown in figure 9.2.

Figure 9.2

1,2,4-trichlorobenzene extraction by direct SPME at 23°C.

Fibre comparison



Repeatability (n = 3) of the automated extraction/injection system for 1,2,4-trichlorobenzene extraction with the PDMS fibre gave RSD's between 2.42-11.01 % and with the PA fibre between 0.15-12.85 %. There was no observable dependence on the RSD's with time, fibre type or chlorobenzene. For both fibres, sorption curves (fig. 9.2) showed an initial rapid partitioning followed by a slower prolonged uptake extending past 120 mins, therefore the true value of Kfs will be slightly higher. As this experiment didn't involve sample agitation, equilibration times were slow. The recoveries for the sorption isotherms at 120 mins indicated that equilibrium is approached without exhaustive extraction occurring.

The partition coefficients (Kfs) calculated at c.a. equilibrium (120 mins) with both fibres are shown in table 9.3.

Table 9.3

A comparison between the partition coefficients of chlorobenzenes for both fibres

	log Krs^{PA}	log Krs^{PDMS}
1,3,5-trichlorobenzene	2.53	2.26
1,2,4-trichlorobenzene	2.78	2.47
1,2,3-trichlorobenzene	2.76	2.54
1,2,4,5-tetrachlorobenzene	2.85	2.62
Pentachlorobenzene	2.97	2.85

The R^2 values for correlation of log Kfs^{PA} and log Kfs^{PDMS} with log Kow were 0.637 and 0.83, respectively (through zero). R^2 values for log Kfs^{PA} and log Kfs^{PDMS} with Henry's constants were 0.86 and 0.83, respectively. Henry's constants were inversely correlated with log Kfs and didn't intercept zero. The results in table 9.3 show that the chlorobenzenes have a higher partitioning in the PA fibre than in the PDMS fibre and increased chlorine substitution is correlated with increased partitioning from water into the fibre and octanol. Increased partitioning in the fibre is also correlated with the decreasing Henry's constants of chlorobenzene (i.e. decreased partitioning into the headspace).

The difference between Kfs^{PA} and Kfs^{PDMS} becomes less as chlorine substitution increases. This indicates a similar partitioning mechanism for pentachlorobenzene in both fibres whilst the other chlorobenzenes may have different interactions with the different fibres i.e. partitioning into PDMS via weak van der Walls forces with stronger electrostatic interactions in the PA fibre. The lower correlation observed between log Kfs^{PA} and log Kow suggests that extraction into PA can not be fully described as a

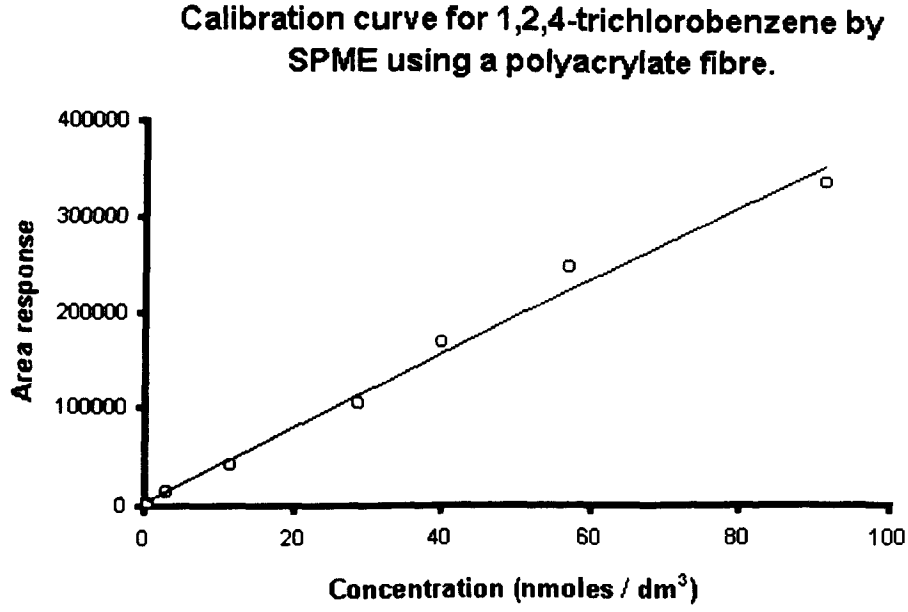
partitioning process. The correlation given in the results with Henry's constants highlights the presence of the headspace in the vial. This factor therefore contributes to the equilibrium amount extracted from the three phase system. Conversely, if the headspace were not present, one wouldn't expect to observe any correlation with Henry's constants.

9.3.1.3 Calibration

When choosing an optimum time for analysis, considerations had to be made concerning accuracy. Taking a time lying in the rapid uptake phase in fig. 9.2 would ultimately rely on the accuracy of the automated system since slight deviations in the extraction time imply larger deviations in amount extracted. To minimise errors a point in the 'lag phase' was chosen as an optimum time (20 mins) to carry out further analysis. Analytes were found to be fully desorbed from the fibre after 10 mins desorption. This time was used as the optimum desorption time to minimise carry over. The PA fibre was used in further studies due to the enhanced extraction compared with the PDMS fibre.

Calibration curves by SPME of 'concentration versus peak area' plotted for each analyte showed linearity between c.a. 0.57-100 nmoles/dm^3. An example of this is given on the following page (fig. 9.3) for 1,2,4-trichlorobenzene. Detection was limited to concentrations above 0.57 nmoles/dm^3. At higher concentrations the line began to plateau.

Figure 9.3



Extractions were repeated 4 times at each concentration. RSD's for 1,2,4-trichlorobenzene were between 13.8 % (0.57 nmoles/dm^3) - 4.9 % (100 nmoles/dm^3) with the PA fibre.

9.3.1.4 Fibre performance

The following page shows the SEM's for the ageing of the PA fibre (figures 9.4-9.6). The fibre showed a visible colour change from white/opaque (at 0 extractions) through to dark brown (with c.a. 400 extractions). It was found that each fibre could carry out up to 200 extractions without detrimental effects on recoveries or sensitivity. After this time the fibre became unreliable and a replacement was required. The SEM's show 'warping' of the fibre surface with extended use, which may explain the decrease in the fibres performance.

Fibre surface viewed by S.E.M

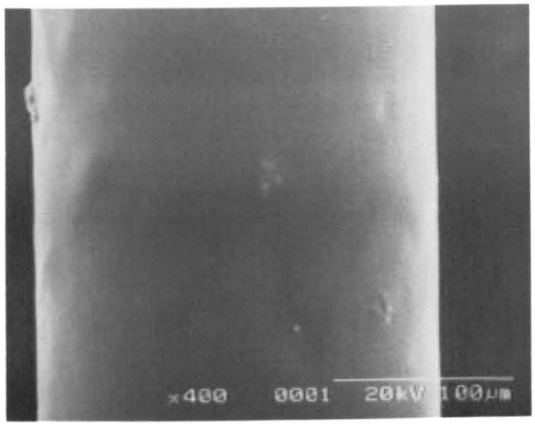


Figure 9.4

0 extractions carried out

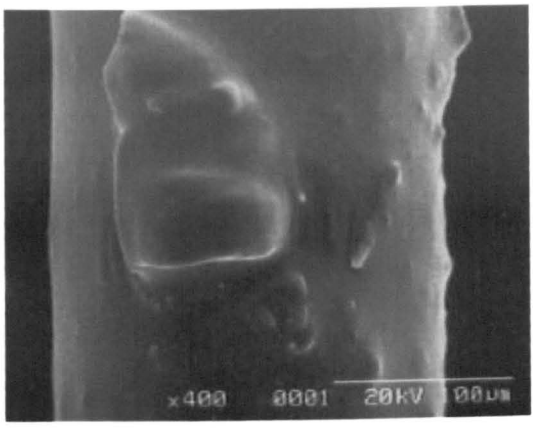


Figure 9.5

After c.a. 200 extractions

Figure 9.6

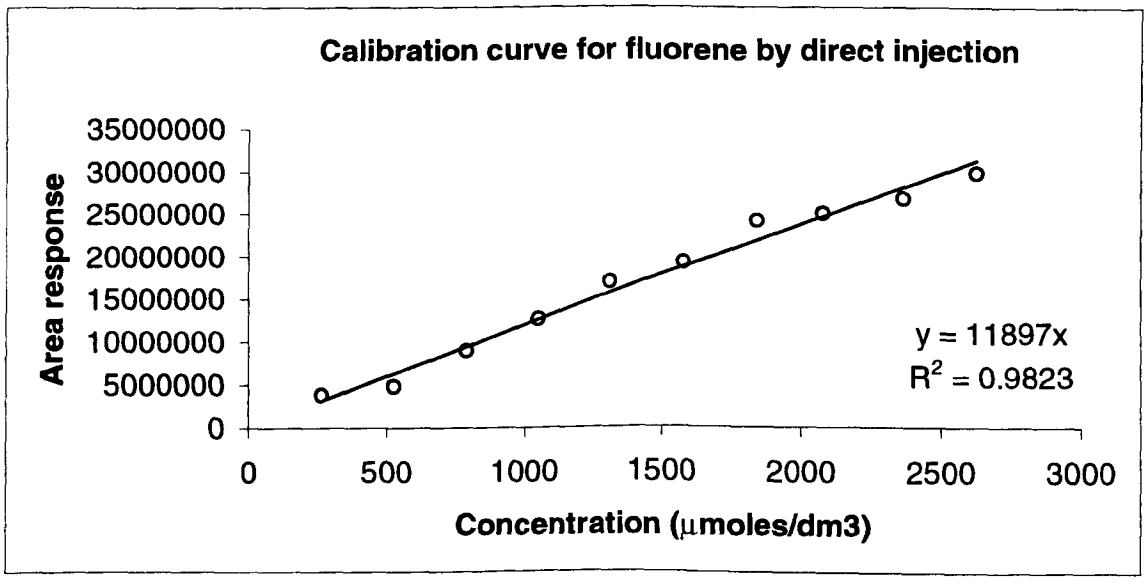
After c.a. 400 extractions

9.3.2 PAH's

9.3.2.1 Direct injection

Calibration curves by direct injection of 'concentration versus peak area' plotted for each analyte showed linearity between c.a. 300-2700 μmoles/dm^3. For example the curve for fluorene is shown in figure 9.7.

Figure 9.7



Repeatability of injection of a single concentration (n = 7) gave RSD's between 11.58-15.00 %.

9.3.2.2 Closed systems

A comparison of extraction recoveries versus time for the extraction of fluorene using both PA and PDMS fibres is shown figure 9.8 below.

Figure 9.8

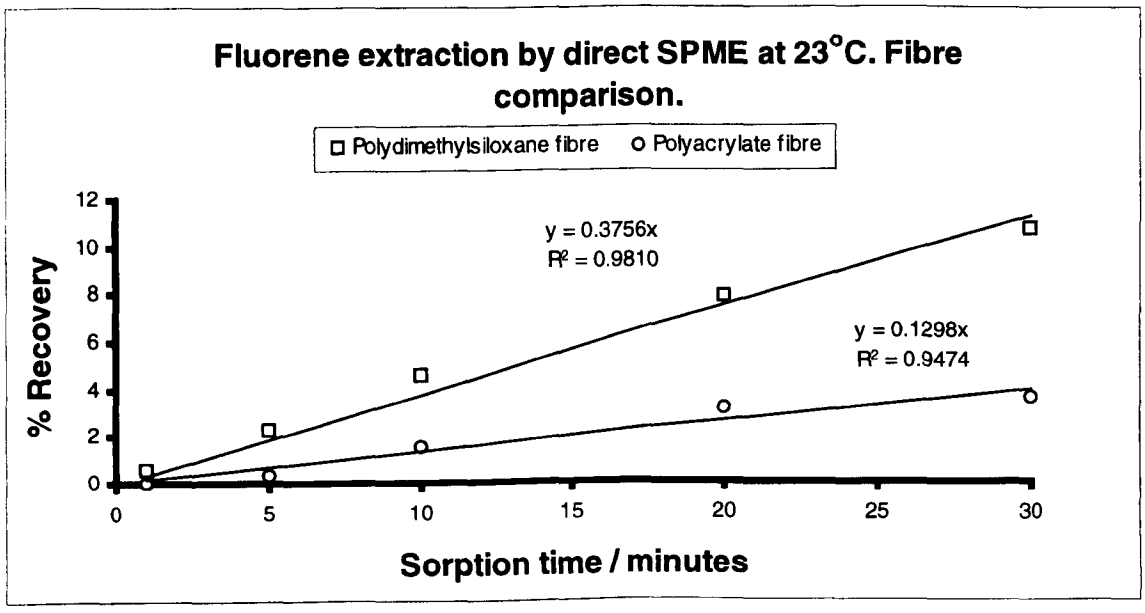


Figure 9.7 shows that on the analysis of 1 ml samples of a c.a. 2 μmoles/dm^3 solution of PAH's in 2 ml sealed vials, equilibrium was not reached for either the PA or PDMS fibre within 30 mins under any set of conditions. Figure 9.8 and table 9.4 show that in general there was a higher extraction of all the PAH's by the PDMS fibre than with the PA coating. This is attributed to the common hydrophobic nature of the PDMS fibre and the PAH's. RSD's (n = 4) for the PDMS fibre ranged between 0.16-2.41 % for direct extraction at 23 °C, 0.53-3.63 % for headspace extraction at 23 °C and 0.21-5.17 % for headspace extraction at 85 °C. The repeatability of the manual extractions of PAH's was comparable with that of the automated method with chlorobenzenes.

The partition coefficients calculated at 30 minutes for both the fibres in each mode of extraction are shown in table 9.4 below.

Table 9.4

Calculated partition coefficients for PAH's

	23 °C direct		23 °C headspace		85 °C direct		85 °C headspace	
	log Kfs^{PA}	log Kfs^{PDMS}	log Kfh^{PA}	log Kfh^{PDMS}	log Kfs^{PA}	log Kfs^{PDMS}	log Kfh^{PA}	log Kfh^{PDMS}
Naphthalene	1.9	2.37	2.33	2.63	1.89	2.25	2.63	2.47
1-methylnaphthalene	1.98	2.43	2.41	2.74	2.38	2.66	2.91	2.8
Fluorene	2.16	2.53	2.37	2.7	2.82	2.99	3.28	3.09
Phenanthrene	2.21	2.58	2.19	2.56	3.1	3.21	3.47	3.29
Anthracene	1.84	2.17	1.91	2.23	3.2	3.24	3.47	3.31
Fluoranthene	2.29	2.59	1.78	2.1	3.34	3.45	3.51	3.4
Pyrene	2.25	2.56	1.69	1.96	3.35	3.47	3.49	3.38

As mentioned in the theoretical section, the equilibrium concentration extracted is independent of the location of the fibre in the system. Therefore at equilibrium, log Kfs should be equal to log Kfh. The differences between these values in table 9.4 is a further indication that equilibrium is not reached within the 30 min extraction time as indicated in figure 9.8. Significant linear correlations were found between log K^{PA} and log K^{PDMS} (table 9.4) and the physical constants given for PAH's in table 9.2. These correlations are summarised in table 9.5 for each set of conditions. 'BP' is the boiling point, 'H' is the Henry's constant, 'Cs' is the solubility and 'Ps' is the vapour pressure. The type of correlation (positive or negative) is also given. The correlation coefficients given for 23 °C direct extraction with both fibres have the result for anthracene omitted. The partitioning of anthracene in both fibres significantly deviated from the correlating sets. For example the R^2 values for the correlation between

Henry's constants and log Kfs^{PA} and log Kfs^{PDMS} are 0.45 and 0.16, respectively when anthracene is included.

Table 9.5

Correlations between partition coefficients and chemical properties of PAH's

		23 °C direct		23 °C headspace		85 °C direct		85 °C headspace	
		log Kfs^{PA}	log Kfs^{PDMS}	log Kfh^{PA}	log Kfh^{PDMS}	log Kfs^{PA}	log Kfs^{PDMS}	log Kfh^{PA}	log Kfh^{PDMS}
log Kow	R^2	0.95	0.87	0.65	0.6	0.92	0.96	0.86	0.91
	correlation	+ ive	+ ive	- ive	- ive	+ ive	+ ive	+ ive	+ ive
BP	R^2	0.92	0.83	0.79	0.75	0.93	0.95	0.86	0.9
	correlation	+ ive	+ ive	- ive	- ive	+ ive	+ ive	+ ive	+ ive
H	R^2	0.95	0.96			0.95	0.95	0.97	0.97
	correlation	- ive	- ive			- ive	- ive	- ive	- ive
Cs	R^2	0.93	0.95			0.89	0.87	0.95	0.91
	correlation	- ive	- ive			- ive	- ive	- ive	- ive
Ps	R^2	0.91	0.94			0.87	0.84	0.93	0.89
	correlation	- ive	- ive			- ive	- ive	- ive	- ive

A summary of the correlations found between partition coefficients of PAH's and chemical characteristics (table 9.5) is given below for each set of extraction conditions.

- At 23 °C for the direct analysis of aqueous solutions of PAH's with both fibres there are significant relationships between the liquid/fibre partitioning coefficient and log Kow, Henry's law constants, solubility, vapour pressure and boiling point. These correlations were only evident once the result for anthracene is discounted. There was a lower partitioning of anthracene which was attributed to its exceptionally higher melting point than the other PAH's (218 compared with phenanthrene 98 °C [15]). Anthracene has the same molecular weight as phenanthrene and possesses three aromatic rings, the only difference is the orientation of the rings. On a molecular level the geometrical ordering of anthracene (i.e. packing arrangement, intermolecular distance, orientation)

must be favoured to allow strong stable crystalline lattice structures to exist in aqueous solution. The association of anthracene molecules in solution is also supported by the solubility data which shows anthracene to be 30 times less soluble than phenanthrene.

Partition coefficients increase with increasing log Kow which is expected within a chemical series due to compounds of increasing hydrophobicity preferring to partition into a hydrophobic phase and having a diminishing hydrophilicity. This is also supported by the correlation with solubility. As solubility decreases, and log Kow increases chemicals prefer to partition into the fibres. Compounds with low boiling points have higher vapour pressures and Henry's constants and so prefer to partition into the headspace above a solution, for this reason it is observed that as boiling points increase and Henry's constants and vapour pressures decrease, partitioning from solution to fibre increases. For example, when comparing naphthalene with pyrene, naphthalene prefers to partition into the headspace at room temperature rather than the fibre due to its lower boiling point and higher Henry's constant, whilst pyrene prefers to partition into the fibre as it has a high boiling point and low Henry's constant. For pyrene another contribution to its high partitioning is its low solubility in water, whilst the opposite is true for naphthalene.

- At 23 °C, the headspace analysis of aqueous solutions of PAH's with both fibres shows that as log Kow and boiling point increase so partitioning decreases. This will mainly be due to boiling point and Henry's constants restricting the heavier PAH's from partitioning into the headspace, whilst the more volatile PAH's have higher air/water partitioning.

The correlation coefficients in this case are lower than in the previous case due to the negative and positive action of individual variables acting on the final result. For example, naphthalene is more soluble in water than pyrene, therefore one would expect pyrene to have a higher partitioning into the headspace due to its hydrophobicity and naphthalene to prefer to stay in water. However the molecular weight and boiling points of these chemicals have a stronger impact on the headspace partitioning than does solubility. It is these factors working against each other which causes a lower correlation under these conditions.

As the partitioning into the headspace is not initially driven by the fiber but by the characteristics of the molecules, there are fewer restrictions once in the headspace for the molecules to partition, and in fact, once in the headspace log Kow becomes meaningless. However there is still a correlation between log Kow and partitioning, so one can assume that there is a relationship between log Kow and log K_{fh} which includes a water air partition coefficient (K_{sh}) or a Henry's constant term. This correlation has been found to be true for the partitioning of volatile compounds from air into leaves [16] which related K_{sh} and Koa (octanol:air) with lipid content of leaves.

Linear relationships were not found for correlation curves relating partitioning with Henry's constants, solubility or vapour pressure. Instead, there is a general increase of these parameters with partitioning followed by levelling of the curves between fluorene and naphthalene.

This suggests that these parameters are more important in determining partition coefficients for the heavier PAH's (phenanthrene to pyrene), whilst they do not restrict

naphthalene, 1-methylnaphthalene or fluorene from entering the headspace or partitioning into the fibre. Under these conditions, naphthalene, 1-methylnaphthalene and fluorene are independent of Henrys law constants as the constants for these compounds are so big.

- At 85 °C, direct analysis shows the same trend as with 23 °C direct analysis with the PDMS fiber having higher partitioning coefficients than the PA fibre reflecting the hydrophobicity of the PAH's and their preference towards the nonpolar fibre. Comparison at the two temperatures also shows evidence of increased partitioning with increased temperature with the exception of naphthalene which has slightly lower partition coefficients. At elevated temperatures a significant portion of the more volatile PAH's will be in the headspace. As Henry's constants, solubility and vapour pressure increase partitioning decreases. As log Kow and boiling point increase so do the partition coefficients.

The direct analysis of aqueous solutions of PAH's at 85 °C with both fibres gives better correlation coefficients than 23 °C direct and 23 °C headspace analysis. As observed at low temperatures, partitioning of anthracene is restricted by melting point and solubility limitations. However at 85 °C there is no discrepancy for anthracene and the correlation between recovery and boiling point is linear for all PAH's analysed. The boiling point of anthracene is one of the only properties which is not dissimilar to that of phenanthrene (340 and 336 °C respectively) and hence at high temperatures the melting point/solubility limitation is overcome and partitioning has a stronger dependence on Henry's constants and boiling point.

If both sample and fibre change temperature between 'To' and 'T' the distribution constant changes according to the following form of the van't Hoff equation (equation 9.1) [17] which describes the influence of temperature on the equilibrium constants Kfs^T and Kfs^{To} at temperatures T and To where ΔH is the standard enthalpy change in J/mol:

$$Kfs^T = Kfs^{To} \exp \frac{-\Delta H}{R} (1/T - 1/To) \qquad \text{(eqn. 9.1)}$$

Where Kfs^{To} is the distribution constant when both fibre and sample are at temperature To (in kelvin), R is the gas constant (8.31 J/K. mol) and ΔH is the molar change in enthalpy of the analyte when it moves from sample to fibre coating and is considered constant for a given analyte over temperature ranges typical for SPME experiments. Hence ΔH can be calculated by measuring Kfs at two different temperatures. The values calculated for the PAH's by direct SPME with the PDMS fibre at 23 °C and 85 °C are : naphthalene (3.9 KJ/mol), 1-methylnaphthalene (-7.5 KJ/mol), fluorene (-15 KJ/mol), phenanthrene (-20 KJ/mol), anthracene (-35 KJ/mol), fluoranthene (-28.1 KJ/mol), and pyrene (-30 KJ/mol).

These values simply express the change in partition coefficients in a different context and show an increase in the molar change in enthalpy between naphthalene and pyrene favouring partitioning of pyrene. It can be seen for naphthalene that ΔH is positive, which reflects the decreased partition coefficient on heating, whilst anthracene has the largest ΔH which reflects the loss of limitations due to melting point considerations.

When the Kfs value for an analyte is greater than 1, the analyte has a lower potential energy in the fibre coating than in the sample, so the analyte partitioning into the

fibre must be an exothermic process (i.e. ΔH is negative). This equation shows that raising the temperature should 'decrease' Kfs. This is due to the increased motion of analytes at high temperatures in the headspace causing higher diffusion rates and hence a lower amount extracted at equilibrium. However, our results do not reflect this, presumably because this effect is only seen for more volatile analytes (as observed with naphthalene) at the temperature employed. For example, Pawliszyn [17] estimated that 5.5 ng of benzene would be extracted from the headspace above a 100 ppb (1.28 μmoles/dm^3) solution at 25 °C whilst only 0.75 ng would be extracted at 90 °C.

- At 85 °C, the headspace analysis of aqueous solutions of PAH's with both fibres gives better correlation coefficients than 23 °C direct and headspace analysis. It is seen that analytes have higher partitioning into the PA fibre than the PDMS fibre in this extraction mode. As log Kow increases so does partitioning into the fibre. The higher correlation between these two variables at 85 °C than at 23 °C in the headspace may be due to water vapour saturation of the headspace leading to a closer estimation of an octanol water system than with direct analysis. Evidence for water vapour in the headspace was observed as condensing water droplets on the fibre. At higher temperatures, the more volatile (higher Henry's constant) compounds have lower partitioning into polymers, (evident from the correlations) due to increased diffusion rates. This is emphasised by naphthalene, which has a lower partitioning at 85 °C than at 23 °C. Analyte loss through the septa is thought to be minimum since large discrepancies in the correlation would expect to be observed.

As temperature increases, the boiling points of heavier PAH's will be less restrictive to headspace partitioning resulting in increased Henry's constants and higher

partitioning into the fibre. However, as described by equation 9.1, the coating/sample distribution coefficient should decrease with an increase in temperature, to some degree counteracting partitioning of heavier PAH's. As observed, this has a greater effect on small volatile compounds which can diffuse more easily in and out of the fibre at higher temperatures thus lowering the equilibrium amount extracted. In the case of elevated temperature, the correlations given in table 9.5 are high when all the PAH's are included. This strongly suggests that at lower temperatures the reason anthracene has a much lower partition coefficient than the other PAH's is due to its very high melting point. This is not a limiting factor at 85 °C as anthracene has a similar boiling point to phenanthrene and therefore correlates well with partitioning.

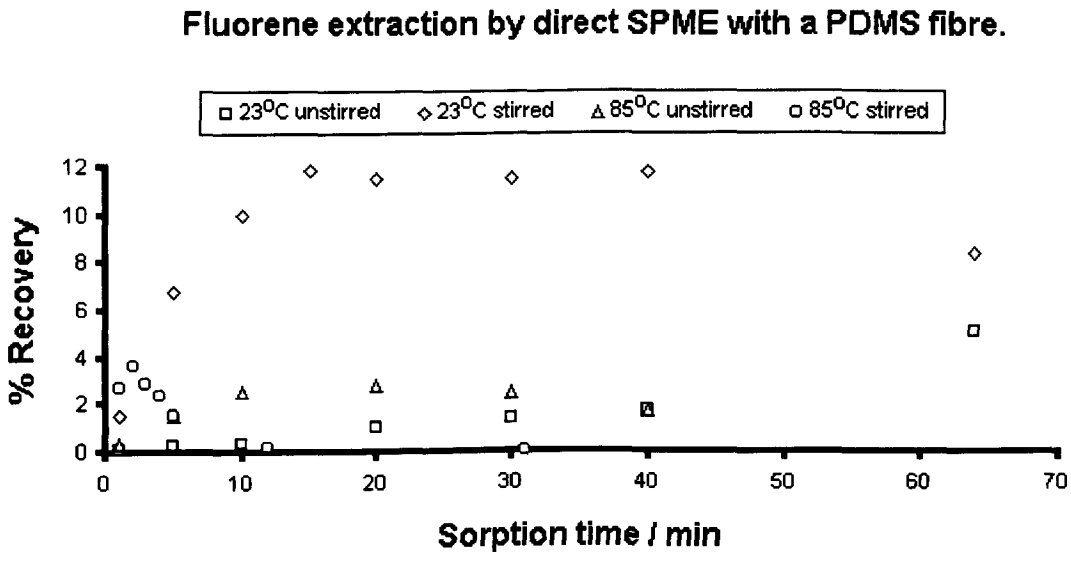
With the optimum conditions used to extract chlorobenzenes, the SPME calibration for PAH's had a linear concentration range between 0.015-2.4 μmoles/dm^3. Both in the case of direct injection and direct SPME, linearity was observed over the range of concentrations measured, and so larger ranges are predicted to be linear.

Like chlorobenzenes, PAH's are non-polar and their extraction should be largely dependent on the weak van der Walls forces governing partitioning. As previously mentioned, larger compounds have a greater amount of van der Walls interactions, and therefore partition coefficients would be expected to increase with increasing molecular size. However, because equilibrium was not reached for the PAH's, the partition coefficients can not be compared with those for chlorobenzenes.

9.3.2.3 Open systems

An example of the direct extraction recoveries from open aqueous systems by SPME under all the conditions used, is shown in figure 9.9 for fluorene.

Figure 9.9



On exposing the fibre directly to the solution in the open systems, analytes partitioned into the fibre, but with time the equilibrium shifted to the open headspace and as a result significant sample loss was observed. The extraction recoveries at elevated temperature were much lower than at room temperature due to evaporative losses.

At room temperature (23 °C) the extraction recovery of the PAH's in the unstirred vials was low and steadily continued to increase past 60 mins. The recovery increased with increasing boiling point of the PAH's (except with anthracene) as observed in sealed systems. Stirring the solutions resulted in equilibration of all the PAH's. However, the rapid equilibration of more volatile analytes was also followed by their rapid loss from the system. This is due to the increased surface area contact and movement of the solution with the headspace facilitating analyte release.

At elevated temperature (85 °C) in unstirred vials, it was found that fluoranthene and pyrene attained equilibrium at c.a. 40 mins whilst naphthalene and 1-methylnaphthalene increased until c.a. 10 mins and were then lost. The extraction of fluorene, phenanthrene and anthracene increased until c.a. 20 mins and then decreased as they were lost from the system. Stirring the solutions resulted in total loss of the volatile analytes between 1-5 mins. With the heavier PAH's initial rapid partitioning occurred until c.a. 5 mins followed by total loss between 5-30 mins.

These experiments show that stirring allows rapid equilibration at room temperature, which, in a closed system would result in enhanced extraction and sensitivity at low concentrations. Agitation in closed systems would also allow analysis of the headspace with out sample loss. Since sorption equilibrium was only observed at 23 °C for stirred systems, partition coefficients were calculated under these conditions for the PAH's at their equilibration times. These values are shown in table 9.6.

Table 9.6

Equilibrium partition coefficients for an agitated solution of PAH's at 23 °C.

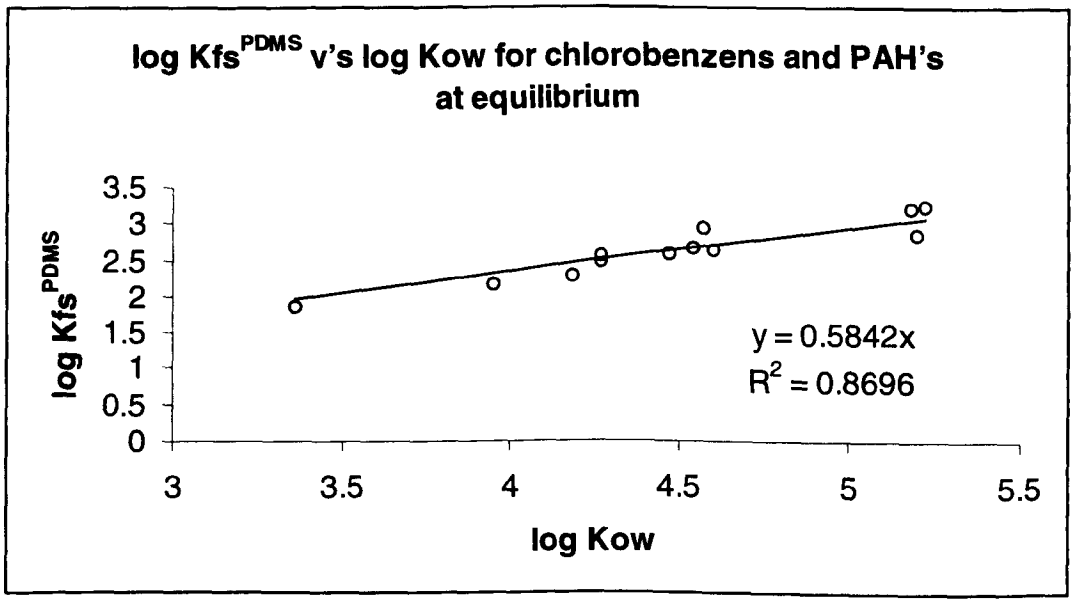
	Equilibrium time/mins	log K_{fs}^{PDMS}
Naphthalene	5	1.86
1-methylnaphthalene	10	2.17
Fluorene	15	2.58
Phenanthrene	40	2.92
Anthracene	64	2.66
Fluoranthene	64	3.22
Pyrene	64	3.21

These values for log K_{fs}^{PDMS} can be used in combination with the equilibrium log K_{fs}^{PDMS} values for chlorobenzenes (table 9.3) and log Kow values to generate a model for the prediction of the partition coefficients of other chemicals.

9.3.3 Prediction of partition coefficients from Kow

Figure 9.10 shows the comparison of log Kow with log K_{fs}^{PDMS} for chlorobenzenes and PAH's at equilibrium at room temperature. The results for chlorobenzenes are from sealed unstirred systems (table 9.3) and PAH's in the stirred open system (table 9.6).

Figure 9.10



Linear regression results in a squared correlation coefficient (R^2) of 0.8696 for the two classes of compounds. The degree of scatter in the results can be shown by taking individual gradient ratios (log K_{fs}^{PDMS} / log Kow). This treatment gives an %RSD = 5.46 and standard deviation = +/- 0.0317 of the gradient. Therefore when comparing

experimental with predicted values of log Kfs^{PDMS} the standard deviation allows a measure to assess the significance of the results. From this correlation the regression equation can be used to predict the partitioning of other compounds based on literature values for their log Kow's. Table 9.7 on the following page shows the predicted Kfs^{PDMS} for several different groups of analytes using literature log Kow values. The available experimental partitioning data is also given for comparison.

Table 9.7

A comparison between predicted and experimental values of Kfs^{PDMS} for different chemical groups

Ref.	Fibre	Compound	log Kow	log K (predicted) S.D. = +/- 0.03	log K (experimental)
11	85 μm PA	2-butoxyethanol	0.83	0.48	0
11	85 μm PA	1-butanol	0.88	0.51	0.05
11	85 μm PA	aniline	0.9	0.53	0.95
11	85 μm PA	3-pentanol	1.21	0.71	0.13
11	85 μm PA	2-nitroaniline	1.37	0.80	1.9
11	85 μm PA	nitrobenzene	1.85	1.08	1.85
11	85 μm PA	1-hexanol	2.03	1.19	0.88
11	85 μm PA	2-nitrotoluene	2.3	1.34	2.55
11	85 μm PA	2-phenylphenol	3.09	1.81	3.37
11	85 μm PA	4-chloro-3-methylphenol	3.1	1.81	2.76
11	85 μm PA	2,4,5-trichloroaniline	3.69	2.16	3.55
11	85 μm PA	2,4,5-trichlorotoluene	4.78	2.79	3.19
12	15 μm PDMS	benz[a]anthracene	5.61	3.28	4.96
12	15 μm PDMS	benzo[a]pyrene	6.44	3.76	4.86
12	15 μm PDMS	chloroform	1.97	1.15	2.6
12	95 μm PDMS	1,1,1-trichloroethane	2.18	1.27	3.4
12	95 μm PDMS	1,2-dichloropropane	2.28	1.33	2.8
12	95 μm PDMS	1,3-dichloropropene	1.41	0.82	1.3
12	95 μm PDMS	1,1,2-trichloroethane	2.18	1.27	3
12	95 μm PDMS	tetrachloroethene	2.6	1.52	3.9
12	95 μm PDMS	bromoform	2.3	1.34	2.8
12	95 μm PDMS	1,1,2,2-tetrachloroethane	2.39	1.40	1.8
13	95 μm PA	pentachlorophenol	5.01	2.93	2.23
13	95 μm PA	2,4,6-trichlorophenol	3.69	2.16	1.78
13	95 μm PA	2,4-dichlorophenol	3.23	1.89	1.67
13	95 μm PA	2,4-dimethylphenol	2.42	1.41	0.96
13	95 μm PA	2-nitrophenol	1.78	1.04	0.57
13	95 μm PA	phenol	1.46	0.85	0.11
13	100 μm PDMS	pentachlorophenol	5.01	2.93	2.57
13	100 μm PDMS	2,4,6-trichlorophenol	3.69	2.16	1.18
13	100 μm PDMS	2,4-dichlorophenol	3.23	1.89	0.66
13	100 μm PDMS	2,4-dimethylphenol	2.42	1.41	0.11
13	100 μm PDMS	2-nitrophenol	1.78	1.04	0.68
13	100 μm PDMS	phenol	1.46	0.85	0.11
14	100 μm PDMS	benzene	2.13	1.24	2.3
14	100 μm PDMS	toluene	2.69	1.57	2.88
14	100 μm PDMS	ethylbenzene	2.84	1.66	3.33
14	100 μm PDMS	m-xylene	3.2	1.87	3.31
14	100 μm PDMS	p-xylene	3.15	1.84	3.31
14	100 μm PDMS	o-xylene	2.77	1.62	3.26

Fig 9.10 shows that at equilibrium there is a strong correlation between log Kow and log Kfs^{PDMS} for PAH's and chlorobenzenes even though the results compare open and closed systems. This correlation supports similar correlation's for specific classes of compounds in the literature [12, 18]. However, table 9.7 shows that when applying these correlation's to predict the partitioning of other classes of compounds, one must be cautious. None of the predicted values are significantly similar to the experimental values (for standard deviation +/- 0.03). Literature values for other compounds are widely variable suggesting that the simple partitioning model can not describe the extraction of different classes of compounds by SPME. The experimental results in table 9.7 show that compounds with different functional characteristics can equally partition between water and octanol but not between water and an SPME fibre.

From table 9.7 it is also clear that there is a tendency for increased partitioning with increased Kow within a chemical series or with a certain phase. However in order to develop a model capable of accurately predicting partitioning, it is evident that the polarity of the phase and chemicals, and types of bonds accounting for 'partitioning', must be considered.

9.4 Summary and conclusions

A fully automated SPME method was developed and optimised for the direct analysis of chlorobenzenes in water. A manual method was developed and the effects of heat and agitation evaluated for extracting PAH's from water. The method developed for chlorobenzenes could be applied to the direct analysis of 'clean' or filtered environmental aqueous samples. The method developed for PAH's could be applied for the direct or

headspace extraction of real aqueous environmental samples. The headspace mode allows an extension of the previous method to analyse samples containing matrix interferences.

The extraction with PA and PDMS fibres was compared. It was found that altering the coating can be used to improve sensitivity. For example, PAH's were favourably extracted by the PDMS fibre whilst chlorobenzenes were favourably extracted by the PA fibre. Diffusion limitations coupled with the ordering of water around the fibre are thought to explain the observed long equilibration times with slow continued uptake for chlorobenzenes and PAH's in non agitated systems.

Unlike the batch equilibration technique (chapter 6), the partition coefficients in these experiments were only found for a single concentration. Although equilibration time with SPME is short in comparison with the batch technique these studies were based around evaluating and optimising SPME as a technique for extraction. Therefore it was decided that it would be impractical to study partitioning at equilibrium over a wide range of concentrations. It was assumed that, at equilibrium, partitioning is constant over a range of concentrations and the rate of attaining equilibrium was independent of concentration. Therefore, 'sorption isotherms' were generated for a series of concentrations under the chosen optimised conditions (and not equilibrium conditions). These optimised conditions allowed quantitative measurements down to levels of c.a. 0.6 nmoles/dm^3 (sub-'part per billion' range) for chlorobenzenes, whilst with PAH's the level was c.a. 15 nmoles/dm^3 ('part per billion' range). Sensitivity could be improved with matrix adjustment or using MS in selected ion monitoring mode. Equilibrium may be reached quickly if sample agitation is involved. Attaining equilibrium in this way

minimises time taken to extract the maximum amount possible and allows higher sample throughput.

Correlations were found between partitioning and log Kow, Henry's constants, solubility, vapour pressure and boiling point for PAH's under different conditions. Correlations were also found between partitioning at equilibrium and Kow and Henry's constants for chlorobenzenes. However the correlation between log Kow and partition coefficients at equilibrium for the PAH's and chlorobenzenes may only be valid for certain chemical series due to different physiochemical descriptors defining the mechanism for sorption or partitioning of chemicals with widely varying functionalities.

Although Kow's are often used in correlations and as a predictive tool with partition coefficients, the correlations are usually based on broad assumptions. For example in the case of soils, it has been noted previously that partitioning can not universally be described for different chemicals by Kow or Koc, and may be more accurately predicted from the aromaticity of humic acids or other contributions from minerals. Likewise, a more environmentally accurate predictor for partitioning than Kow in living organisms may be the partition coefficient between water and lipid phases [2-3, 5-6, 19-20]. In future applications, Kfs may be used to predict partitioning of chemicals in key environmental phases including soils and lipids by relating partitioning to the physical and chemical properties of the analyte, fibre and environmental phase.

With rapid advancement in SPME, the economics of using sorbent coated fibres which can be regenerated many times is an attractive alternative to traditional and some modern methods of extraction.

9.5 References

1. C.G. Choudhry and O. Hutzinger, *Environ. Sci. Technol.*, **18** (1984) 235.
2. W.Y. Shiu and D. Mackay, *J. Chem. Eng. Data*, **42** (1997) 27.
3. W.de Wolf, J.H.M. de Bruljn, W. Selnen and J.L.M. Hermens, *Environ. Sci. Technol.*, **26** (1992) 1197.
4. S.H. Yalkowsky and S.C. Valvani, *J. Pharm. Sci.*, **69** (1980) 912.
5. A.P. Van Wezel, G. Cornelissen, J. Kees, V. Miltenburg and A. Opperhuizen, *Environ. Toxicol. Chem.*, **15** (1996) 203.
6. M.R. Mortimer and D.W. Connell, *Aust. J. Mar. Freshwater Res.*, **44** (1993) 565.
7. M. Alaee, R.M. Whittal and W.M.J. Strachan, *Chemosphere.*, **32** (1996) 1153.
8. S. Motlagh and J. Pawliszyn, *Anal. Chim. Acta*, **284** (1993) 265.
9. J. Porschmann, F.D. Kopinke, M. Remmler, K. Mackenzie, W. Geyer and S. Mothes, *J.Chromatogr. A*, **750** (1996) 287.
10. R.W. Walters and R.G. Luthy, *Environ. Sci. Technol.*, **18** (1984) 395.
11. W.H.J. Vaes, C. Hamwijk, E.U. Ramos, H.J.M. Verhaar and J.L.M. Hermens, *Anal. Chem.*, **68** (1996) 4458.
12. J.R. Dean , W.R. Tomlinson, V. Makovskaya, R. Cumming, M. Hetheridge and M. Comber, *Anal. Chem.*, **68** (1996) 130.
13. K.D. Buchholz and J. Pawliszyn, *Anal. Chem.*, **66** (1994) 160.
14. D.W. Potter and J. Pawliszyn, *J. Chromatogr.*, **625** (1992) 247.
15. S.H. Yalkowski, *J. Pharm. Sci.*, **70** (1981) 971.
16. M.H. Hiatt, *Anal. Chem.*, **70** (1998) 851.
17. J. Pawliszyn, In '*Solid Phase Microextraction, Theory and Practice*'; Wiley - VCH, Inc. John Wiley & Sons, Inc., 605 Third Avenue, New York, NY (1997).

18. C.T. Chiou and D.E. Kile, *Environ. Sci. Technol.*, **28** (1994) 1139.

19. W.E. Pereria, C.E. Rostad, C.T. Chiou, T.I. Brinton and I.I. Barber, *Environ. Sci. Technol.*, **22** (1988) 772.

20. L.P. Burkhard, B.R. Sheedy, D.J. McCauley and G.M. DeGraeves, *Environ. Toxicol. Chem.*, **16** (1997) 1677.

Chapter 10.0

Quantitative aspects of SPME: Headspace extraction of solids

10.1 Introduction

Based on the results of previous studies (chapter 9.0), to afford maximum recoveries the 85 μm polyacrylate fibre was used to extract chlorobenzenes and the 100 μm polydimethylsiloxane fibre was used to extract PAH's.

After initial use of screening and identification of compounds present in several solid matrices (chapter 8.0) the aim of this study was to develop a method for quantifying the levels of chlorobenzenes and PAH's present in certified reference materials, contaminated land samples and diesel exhaust particles. Optimisation included evaluating the effects on extraction recoveries of heating and wetting the samples. The use of different quantification methods was also evaluated.

10.2 Experimental

10.2.1 Chlorobenzenes

The two soils obtained from the 'European Commission, Community Bureau of Reference (BCR)', had little information supplied concerning the levels of chlorobenzene contamination. These soils were known to be heavily contaminated with many chlorophenols and also high levels of lindane (hexachlorocyclohexane) and its isomers. The documentation received regarding these materials only gave levels for the range of contamination of 1,2,3-trichlorobenzene (5-50 mg/kg) and pentachlorobenzene (2-20 mg/kg) on the sandy soil. The

clay soil was known to be contaminated to a much higher degree than the sand. Based on this limited information, the BCR reference materials were treated as surrogates for experimental optimisation by SPME and for comparison with different forms of extraction.

A manual sampling protocol was optimised for headspace analysis of chlorobenzenes from the reference materials. The polyacrylate fibre was used exclusively in these extractions. The sandy soil was chosen to carry out most initial optimisation studies. GC-MS was used to separate and quantify the extracted analytes.

Quantification was carried out by GC-MS. The instrumentation used in the experiments was described in chapter 3. Sample heating was carried out using the water bath apparatus shown in figure 8.1.

10.2.1.1 The effects of temperature and water on extraction

Primarily, sandy soil (c.a. 0.02 g) was weighed into 2 ml vials and wetted with 0.1 ml water. Samples were heated at different temperatures and the headspace extracted for different lengths of time to asses the effect of these conditions on analyte equilibration (inferred from the detector response). All sorption curves were carried out over times ranging from 0-50 mins.

To investigate the effect on extraction of adding water to the sandy soil, a small mass of the soil (0.02 g) was placed in 2 ml vials and 0.1 ml water was added. A set of vials were

also prepared for analysis which contained sandy soil with no added water. A fixed extraction time of 20 mins was chosen based on the responses from the previous experiment. Both sets of vials were heated at different temperatures (23, 40, 60 and 80 °C) and the headspace extracted by SPME.

10.2.1.2 The effect of water/soil ratio on extraction

The effect of wetting the soil was found to enhance extraction and so the water/soil ratio was considered an important factor to investigate when optimising the method. Therefore the volume of water required to extract the maximum amount of chlorobenzenes under a constant set of conditions was determined. Ideally this must be determined for any solid matrix extractives requiring quantification. However, the effect of water/soil ratio on the extraction of chlorobenzenes from the clay soil was only investigated. Due to the high levels of chlorobenzenes on the clay soil it was assumed that in this case, the alteration of the water/soil ratio would have the greatest effect on extraction recoveries from this soil.

Before extracting the headspace, a fixed volume of water (0.2 ml) was added to varying masses of soil. The soil solutions were heated to 80 °C and the headspace extracted for 20 mins by SPME. The extracted amount was quantified by external calibration with '0.2 ml' water standards of varying concentration.

10.2.1.3 Methods of quantification

In order to be able to quantify the levels present in the soil, two methods of external calibration were investigated. The method of internal standard addition was also evaluated.

- **External calibration method 1. 'Solid standards'.**

The problem of quantifying the amount of the target analytes actually present in the soil is the main drawback. In order to achieve this one must ideally use a series of artificially spiked solid standards for external calibration which have an identical matrix to the sample.

Soil matrices vary greatly in character from different locations (i.e. organic carbon content, cation exchange capacity, mineral content) so choosing an identical clean matrix to artificially spike and use as an external standard is virtually impossible. Even if a suitable matrix could be found, complications would arise concerning the loss of recovery with time after spiking due to sorption interactions with the chosen matrix.

It was initially decided to minimise these complications by choosing Celite to simulate soil as an inert matrix. It was believed that Celite would allow a maximum extraction recovery. The use of this simple matrix was based on the assumption that the amount extracted from Celite and soil under the same conditions would be relative to the conditions used and not to the matrix. However, strongly sorbed species in soil may not be released to the same extent as with Celite and so these fractions can't be quantified. At best,

the use of Celite as a standard would give an underestimation of the amounts actually sorbed to the soil. Celite standards were prepared by slurry spiking with different concentrations of chlorobenzenes in DCM.

- **External calibration method 2. 'Aqueous standards'.**

Wetting the soil was previously found to increase the extraction of all the chlorobenzenes. Adding water to the soil also allows the advantage of treating the soil as a solution. Therefore external water standards (of the same volume as that of the water added to the soil) can be extracted under the same conditions to quantify the levels of chlorobenzenes present on the soil. The use of these standards is also based on the same assumptions regarding extraction recoveries from Celite. It also does away with the requirement of choosing and spiking suitable solid standards which may have unpredictable complex interactions with analytes over time.

From the results of the water/soil studies with clay soil it was assumed that due to the much lower levels of chlorobenzenes in the sandy soil, a lower water/soil ratio could be used with sandy soil to affect maximum recovery.

Chlorobenzenes were extracted from sand and clay samples using two sets of water/soil ratios and quantified using 'GC-MS' (details given in chapter 3.0). These ratios were 0.01 g sand/0.15 ml water (ratio = 15) and 0.003 g clay/0.15 ml water (ratio = 50).

The vials were lowered into a water bath and maintained at a constant temperature of 80 °C. The 85 μm thickness polyacrylate fibre was exposed to the headspace above the sample matrix for 20 mins to allow partitioning of the analytes between sample and fibre. A desorption time of 10 mins was used based on previous studies. The injection port was at 280 °C and operated in splitless mode at the time of injection (split at 11 mins). The detector temperature was 300 °C.

- **Internal standard addition method with aqueous standards.**

The validity of using standard additions was also investigated on both soils. Water standards of fixed volume were applied with increasing concentration directly to each reference material prior to extraction. This method of quantification was repeated three times with both sandy and clay soils.

For internal standard addition/calibration, soils of the same mass (c.a. 0.01 g sand, c.a. 0.003 g clay) were weighed into 2 ml vials and wetted with 0.15 ml of the chlorobenzene solutions of varying concentrations.

10.2.1.4 Soxhlet, shake flask and accelerated solvent extractions

Although conditions (i.e. shorter extraction time) can be optimised to enable larger sample masses to be used, the SPME headspace method was developed for use with 2 ml autosampler vials and only required a small sample amount. In order to determine whether

the reference materials were homogenous and small sample sizes could be representative of the bulk, larger amounts of the clay and sand BCR materials were also extracted by shake flask, ASE and Soxhlet using DCM as a solvent. This also allowed direct comparison of the extraction efficiency of the SPME technique.

An internal standard (1-methylnaphthalene), which was not present on the soil and didn't interfere with any of the peaks, was added to each extract. The extracts from these techniques were filtered and the solvent evaporated to dryness by rotary evaporation, then made up to 1 ml in DCM. The internal standard accounted for sample losses on filtration. However, as the Henry's constant of 1-methylnaphthalene is much lower than that of chlorobenzenes it may only account, in part, for losses on evaporation. Since MS detection was used, a radiolabelled chlorobenzene analogue of one of the quantified species should ideally be used to realistically account for the losses on filtration and evaporation.

ASE was also used for hot water extraction under different conditions, followed by SPME of the water extracts. For ASE-DCM or ASE-water extracts three sets of extraction conditions were used with the ASE which are shown in table 10.1. The water extracts (1 ml taken) were analysed by SPME headspace under the same conditions used previously, and quantified by external calibration using aqueous solutions of chlorobenzenes. All the DCM extracts were quantified by direct injection with external calibration solutions prepared in DCM with the same amount of internal standard (1-methylnaphthalene) added before analysis.

Table 10.1

The different extraction conditions employed with the 'ASE'

	Accelerated solvent extraction conditions		
	Set 1	Set 2	Set 3
Pressure (psi)	1100	2000	2000
Temperature (^{o}C)	85	200	200
Heat (mins)	5	5	30
Static (mins)	5	5	30
Flush volume (%)	100	100	100
Purge time (seconds)	200	200	200
Cycles	1	1	1

Soxhlet and shake flask extractions were carried out for 24 hours. Soxhlet extraction involved refluxing heated DCM through the soil matrix, whilst shake flask involved shaking the soil in unheated DCM.

10.2.2 PAH's

The reference material supplied by the 'Laboratory of the Government Chemist (LGC 6138)' was a coal-carbonisation site soil and contained mainly metals and PAH's. Sulphur content was very high (45,900 mg/kg) compared to PAH's which ranged from 15.3 mg/kg (fluorene) to 118 mg/kg (fluoranthene). However the legal notice which accompanied the document stated that there was no guarantee with respect to accuracy of the information given. Real samples of industrially contaminated soils were provided by Northumbrian Water and diesel exhaust pipe soot was collected from a nearby car park.

The method developed to extract and quantify chlorobenzenes was applied to quantifying the levels of PAH's extracted from the reference material and other matrices by GC-MS. The results for the reference material were compared with the certified values. The PDMS fibre was used exclusively with these extractions and due to the fibres suspected thermal instability the injection port temperature was lowered to 250 °C.

10.2.2.1 Quantification of PAH's in a reference material

The levels of PAH's were extracted from, and quantified in the reference material 'LGC 6138' by using two methods.

- The same conditions for SPME of chlorobenzenes from the clay soil were used. A small mass of soil (c.a. 0.003 g) was weighed into a 2 ml vial and 0.15 ml of water added. The soil was heated to 80 °C for 20 mins whilst extracting the headspace.

- A larger mass of soil (c.a. 1 g) was weighed into a 50 ml vial and 1 ml water was added. A magnetic stir bar was added and the vial was sealed. The slurry was magnetically stirred whilst heating in a water bath and the headspace extracted for 20 mins.

Five repeat extractions were carried out on the CRM (LGC 6138) for each of these methods.

10.2.2.2 Quantification of PAH's in environmental matrices

Semi quantitative analysis was carried out on all the soils and the exhaust soot in order to decide which mass to analyse to avoid fibre overloading and to remain within the range of the external calibration solutions. The same wetting volume, heating conditions and sampling time were then used for all the matrices as used for the reference materials. The mass used for the quantitation of each matrix was 0.1 g 'exhaust soot', 0.01 g 'contaminated soil 1', 0.2 g 'contaminated soils 2 and 3', and 0.4 g 'garden soil'. Each soil was weighed into 2 ml vials and a constant 0.15 ml of water was added before extraction. The vials were heated to 80 ^{o}C and the headspace extracted for 20 mins.

10.3 Results and discussion

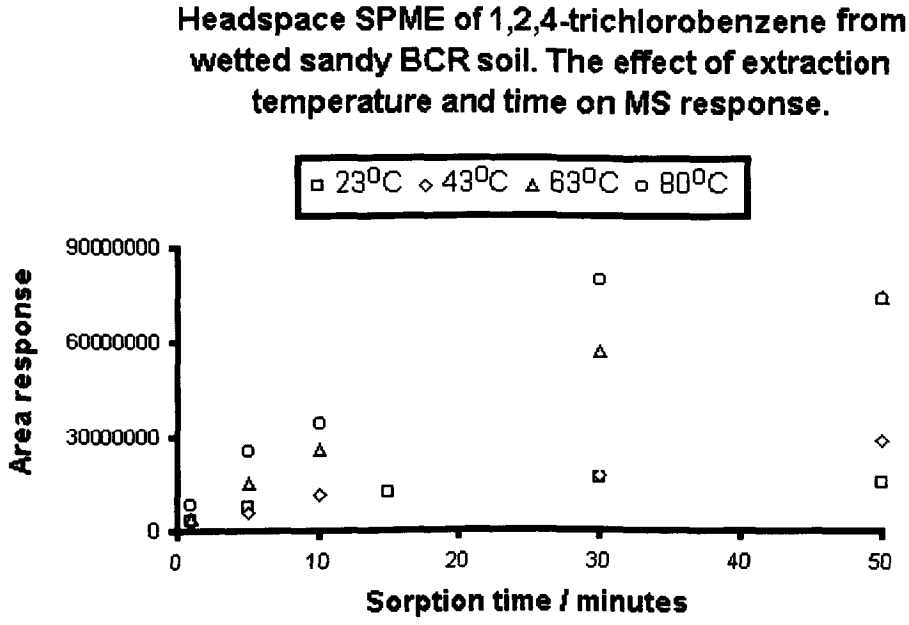
10.3.1 Chlorobenzenes

Typical chromatograms for the separation of the chlorobenzenes extracted from the sandy and clay soils have been shown previously (figures 8.7-8.8) .

10.3.1.1 The effects of temperature and water on extraction

Figure 10.1 shows the results for headspace extraction of 1,2,4-trichlorobenzene from wetted sandy soil at different temperatures for differing lengths of time. The same trend occurred with all chlorobenzenes extracted.

Figure 10.1



The sorption curves in fig. 10.1 for wetted sandy soil at 20 and 40 °C increased almost linearly with time with little difference in the amount extracted between temperatures. At 60 and 80 °C the curves showed an initial higher partitioning followed by a slow prolonged uptake extending past 50 min.

Figure 10.2 on the following page compares the results for 20 minute extractions from dry soil and soil wetted with 0.1 ml of water with increasing temperature. The same trend occurred with all chlorobenzenes extracted.

Figure 10.2

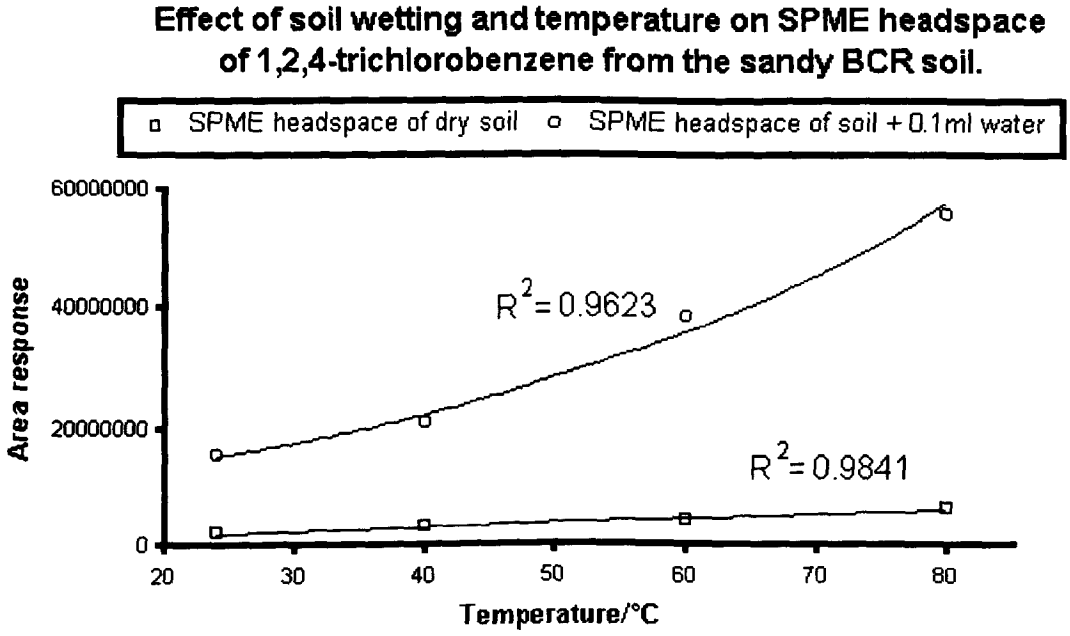


Figure 10.2 shows there is a linear increase in extraction with temperature for the dry soil ($R^2 = 0.9841$) whilst wetted soil showed deviations from linearity at higher temperatures together with a much higher extraction ($R^2 = 0.9623$). Figure 10.2 also shows that when water is added to dry soil there is a ten fold increase in extraction from the headspace at room temperature and a further five fold increase in extraction on heating the wetted sample at 80 °C. This trend was evident for all the chlorobenzenes.

The addition of water introduces a solvent extraction mechanism which firstly increases the contact surface area of the soil bound chlorobenzenes with the extraction medium and secondly displaces and solvates the chlorobenzenes from the soil surface which are weakly bound by hydrophobic interactions.

Raising the temperature also enhances the extraction firstly by increasing the solvating power of water by lowering its viscosity. This will also enable enhanced penetration of the solvent into the soil's microporous structure. This in turn increases solubility and molecular diffusion of chlorobenzenes from the soil matrix into the bulk water.

Increasing the temperature will also speed up mass transport into the headspace by increasing the vapour pressure of the chlorobenzenes. Raising the temperature also enhances the extraction of chlorobenzenes by overcoming the activation energy of desorption from high energy sorption sites on the soil.

The coupling of water with heat may also alter the soils structure by swelling the humin-kerogen polymeric complexes associated with clay components in soil. These polymers are thought to interact extensively with organic compounds in soils. Swelling of polymers by using selected solvents is a well known phenomenon [2] and may further facilitate the release of chlorobenzenes.

10.3.1.2 The effect of water/soil ratio on extraction

The effect of increasing the amount of water added to clay BCR soil on the extraction of chlorobenzenes is highlighted in figure 10.3 for 1,2,4-trichlorobenzene. The same trend occurred with all chlorobenzenes extracted.

Figure 10.3

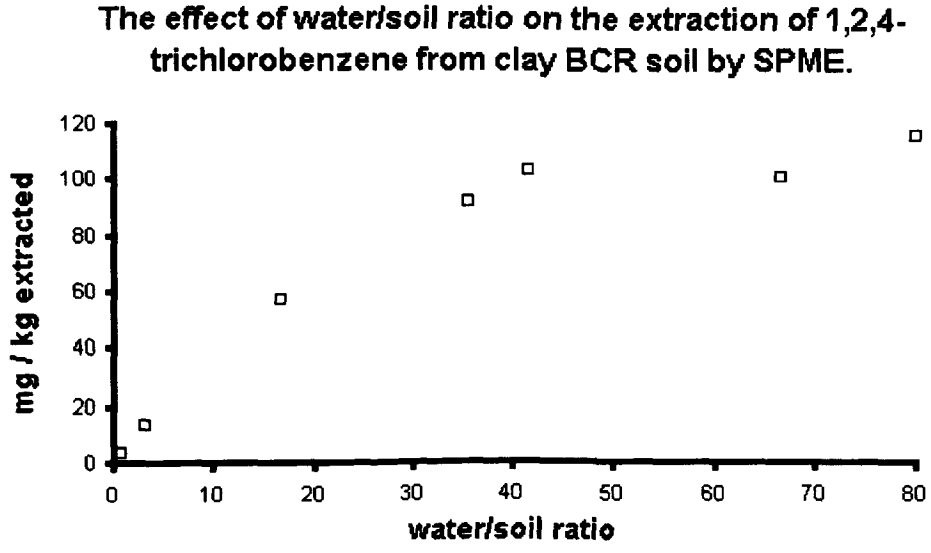


Figure 10.3 shows that increasing the water/soil ratio results in increased extraction recoveries from clay BCR soil until a ratio of c.a. 40, after which no further extraction was observed. Boesten [3] reviewed about 30 measurements on the influence of solid/liquid ratio on pesticide sorption isotherms and found that there was a considerable decrease in sorption as the amount of water increases. This supports our finding that the addition of increased amounts of water to the sample matrix shows higher extraction recoveries of chlorobenzenes from clay soil.

10.3.1.3 Quantitation of chlorobenzenes in the reference materials

- **The use of external calibration with Celite standards**

Chlorobenzenes were found to irreversibly bind to Ceite standards leading to inefficient recoveries. Therefore the use of Celite was not continued.

- **External calibration with standard solutions and standard additions**

Figures 10.4 (sand) and 10.5 (clay) on the following page show an example of the determinations for quantitation by standard addition. The graphs show the concentration added against the detector response for 1,2,4-trichlorobenzene. Where the lines of the standard addition curves intercept the x-axis, the level of 1,2,4-trichlorobenzene on each soil can be inferred. The average results for repeat determinations are tabulated in table 10.2.

Figure 10.4

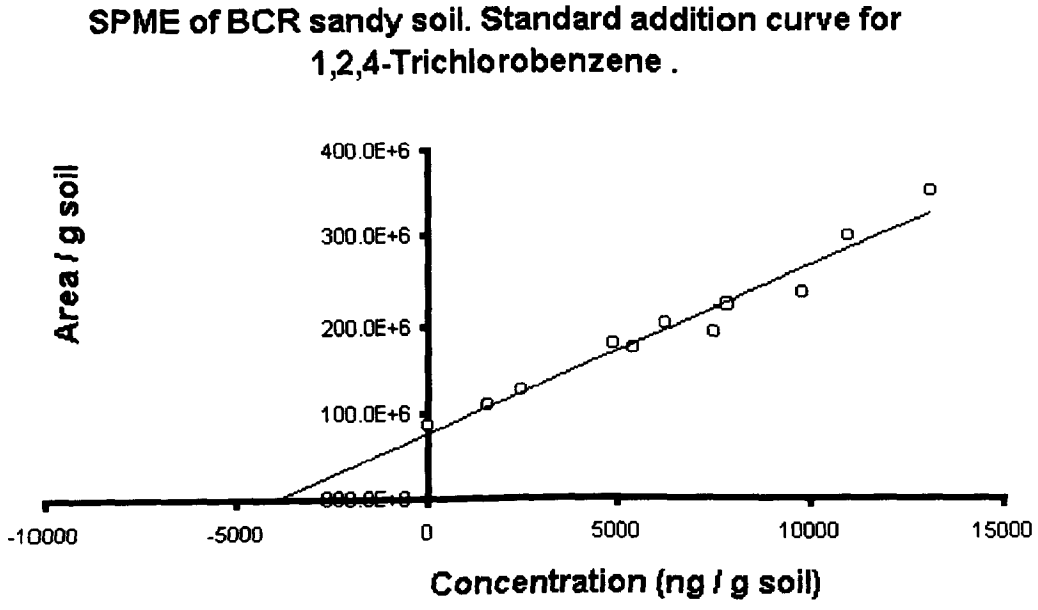
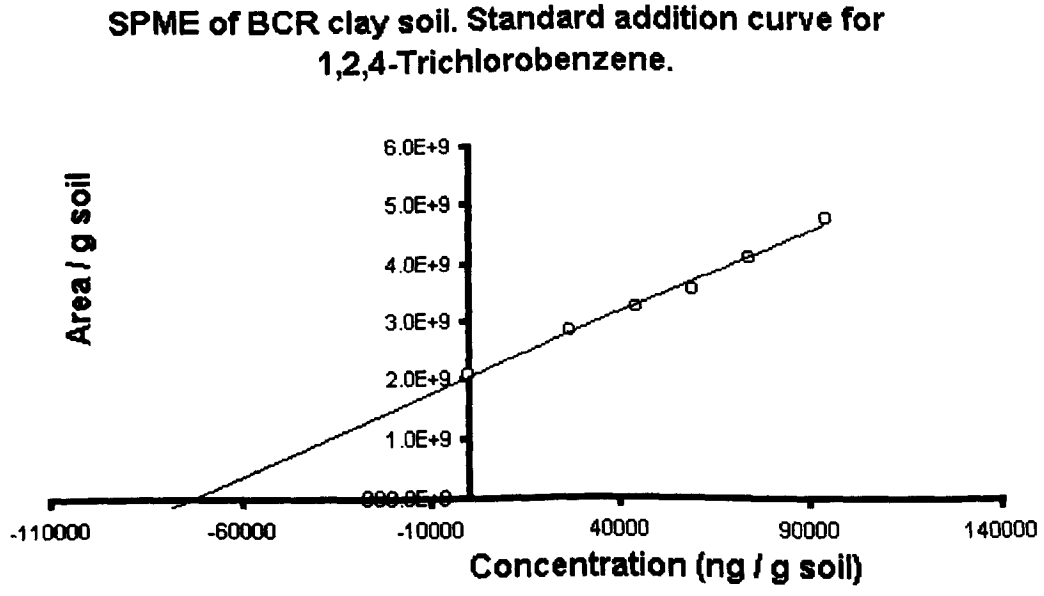


Figure 10.5



Quantification by GC-MS of chlorobenzenes using external standards for both clay and sandy soils are given in table 10.2 on the following page. The levels for 1,2,3-trichlorobenzene and pentachlorobenzene supplied by BCR for the actual levels on the sandy soil were 5-50 mg/kg and 2-20 mg/kg respectively. Table 10.2 compares our values with the levels supplied by BCR and the values obtained by Santos et al. [1] using a similar SPME method with the sandy soil. Standard deviations (from % RSD's) of the results in table 10.2 for both quantification procedures were used to test whether the mean extraction values (for sand and clay) were significantly similar. This involved a t-test for P = 0.05 with the equations 10.1 and 10.2 below [4]:

$$s^2 = \{(n_1 - 1)\, s_1^2 + (n_2 - 1)\, s_2^2\} / (n_1 + n_2 - 2) \qquad \text{eqn. 10.1}$$

$$t = \{(\text{average}_1 - \text{average}_2) / s\} \times \text{square root}\,(1 / n_1 + 1 / n_2) \qquad \text{eqn. 10.2}$$

where 's' is the standard deviation, 'n' is the number of replicates and 't' has $n_1 + n_2 - 2$ degrees of freedom. The critical values of 't' (for P = 0.05) were 2.78 for sand samples (4 degrees of freedom) and 2.31 for clay samples (8 degrees of freedom). The 't' values calculated for significance comparison between quantification results in table 10.2 for sand and clay soils are given in table 10.3.

Table 10.2

Results for repeat analyses of both sandy and clay soils by GC-MS

	Sandy soil	Sandy soil	Sandy soil	Sandy soil	Clay soil	Clay soil
	Standard additions Average mg/kg n=3 (RSD %)	External calibration Average mg/kg n=3 (RSD %)	BCR values mg/kg	External calibration Reference [1] mg/kg	Standard additions Average mg/kg n=3 (RSD %)	External calibration Average mg/kg n=7 (RSD %)
Water/soil ratio	15	15		0.1	50	50
1,3,5-trichlorobenzene	**0.18** (48.94)	**0.20** (29.8)			**1.54** (14.64)	**1.04** (24.35)
1,2,4-trichlorobenzene	**4.35** (28.2)	**4.6** (25.5)			**82.12** (13.47)	**98.62** (22.79)
1,2,3-trichlorobenzene	**3.04** (21.89)	**2.67** (8.7)	5.0 - 50.0	0.59	**8.98** (19.41)	**9.12** (21.06)
1,2,4,5-trichlorobenzene	**5.83** (29.89)	**5.37** (19.2)			**17.27** (24.29)	**10.67** (23.38)
Pentachlorobenzene	**0.72** (40.15)	**0.47** (37.2)	2.0 - 20.0	1.42	**16.16** (42.58)	**23.87** (25.35)

Table 10.3

't' values calculated from quantification results in table 10.2 for sand and clay soils

	Sandy soil	Clay soil
	t' (P = 0.05, 4 degrees of freedom)	t' (P = 0.05, 8 degrees of freedom)
1,3,5-trichlorobenzene	-0.22	1.40
1,2,4-trichlorobenzene	-0.17	-0.56
1,2,3-trichlorobenzene	0.61	-0.05
1,2,4,5-trichlorobenzene	0.26	1.51
Pentachlorobenzene	0.85	-0.85

The observed values of 't' in table 10.3 are all lower than the critical 't' values taken [4]. This means that the results found using both standard additions and external standard calibration for both sand and clay soils in table 10.2 are not significantly different (for P = 0.05).

10.3.1.4 Soxhlet, shakeflask and accelerated solvent extractions

Table 10.4 on the following page shows the average results and RSD's of Soxhlet, shakeflask and accelerated solvent extractions of sand and clay soils. The solvent and volume used in all the extractions is also given.

The areas of the internal standard 1-methylnaphthalene in the external standards were comparable with those in the extracts showing minimum loss due to filtration or evaporation.

Table 10.4

Chlorobenzenes extracted (mg/kg) from sand and clay BCR soils using ASE, shake flask and Soxhlet.

	Accelerated solvent extractions					Shake flask extraction	Soxhlet extraction
Conditions Solvent (volume)	**Set 1** DCM (25ml)	**Set 2** DCM (25ml)	**Set 3** DCM (25ml)	**Set 1** Water (25ml)	**Set 3** Water (25ml)	DCM (80ml)	DCM (180ml)
Sand (CRM 529)	**Average n = 2** (results)	**Average n = 3** (% RSD)	**Average n = 2** (results)	**Average n = 5** (% RSD)	**Average n = 3** (% RSD)	**Average n = 3** (% RSD)	**Average n = 4** (% RSD)
1,3,5-TCB	**0.045** (0.048, 0.042)	**0.14** (37.23)	**0.12** (0.1, 0.13)	**0.007** (20.31)	0	**0.053** (5.85)	**0.019** (14.96)
1,2,4-TCB	**3.65** (3.46, 3.84)	**5.55** (3.19)	**5.78** (5.72, 5.84)	**0.39** (12.57)	**0.1** (52.31)	**2.62** (7.9)	**1.05** (6.83)
1,2,3-TCB	**0.59** (0.54, 0.64)	**0.93** (9.78)	**1.09** (1.12, 1.06)	**0.1** (28.64)	**0.026** (74.82)	**0.37** (4.89)	**0.21** (10.71)
1,2,4,5-TetCB	**1.03** (1.12, 0.93)	**1.96** (13.3)	**2.1** (2.24, 1.95)	**0.1** (40.44)	**0.036** (9.3)	**0.96** (25.17)	**0.71** (7.4)
PentaCB	**1.2** (1.46, 0.94)	**2.37** (36.18)	**2.93** (3.45, 2.4)	**0.054** (63.99)	**0.047** (76.62)	**1.46** (26.26)	**1.23** (21.79)
Clay (CRM 530)	**Average n = 2** (results)	**Average n = 2** (results)	**Average n = 2** (results)	**Average n = 5** (% RSD)	**Average n = 3** (% RSD)	**Average n = 3** (% RSD)	**Average n = 4** (% RSD)
1,3,5-TCB	**0.104** (0.105, 0.101)	**0.067** (0.077, 0.055)	**0.1** (0.09, 0.11)	**0.057** (23.68)	**0.11** (17.18)	**0.1** (8.59)	**0.097** (6.57)
1,2,4-TCB	**26.57** (26.45, 26.69)	**19.31** (19.22, 19.39)	**18.75** (18.62, 18.88)	**8.67** (24.96)	**27.84** (11.11)	**28.58** (2.08)	**30.82** (2.07)
1,2,3-TCB	**4.11** (4.17, 4.04)	**3.23** (3.11,3.34)	**3.21** (3.25, 3.17)	**1.25** (23)	**5.62** (10.58)	**4.91** (2.79)	**5.1** (0.81)
1,2,4,5-TetCB	**5.4** (5.45, 5.35)	**4.51** (4.42, 4.61)	**5.09** (5.41, 4.77)	**3.59** (10.37)	**18.12** (8.25)	**6.07** (5.6)	**6.89** (4.44)
PentaCB	**2.13** (1.85, 2.4)	**2.16** (2.26, 2.05)	**2.41** (2.61, 2.21)	**0.53** (29.18)	**2.63** (6.82)	**2.1** (11.49)	**2.49** (4.54)

From figure 10.3 it can be seen that the water/soil ratio to achieve maximum recovery of 1,2,4-trichlorobenzene from the clay soil (c.a. 100 mg/kg) is c.a. 40. Due to the lower levels on sandy soil a lower water/soil ratio was chosen to extract the soil.

In a similar experiment to ours, Santos et al. [1] also analysed the sandy BCR soil by SPME and found an increase on extraction with the addition of water. They observed a maximum extraction at a very low soil/solution ratio (0.1). Due to the very low levels of chlorobenzenes on the sandy soil compared to the clay soil this suggests that soil/water ratio is not as important for the extraction of low levels of chlorobenzenes from soils. The reason for this may be that lower concentrations are soluble in smaller volumes of water than high concentrations. When the concentration is low, most of the analyte can be extracted from the matrix due to low solubility limitations in solvent, and thus, negating the sorption interactions of different matrices, one would expect that different extraction methods may yield comparable results when analysing low levels of pollution. Conversely, methods which use low soil/solution ratios to analyse higher levels of pollution may yield underestimated or fluctuating results. For higher concentrations the solvent/soil ratio may play an important role in determining the amount extracted due to solubility of chemicals decreasing in lower amounts of solvent. A model named the 'hot ball model' has been proposed by Bartle et. al. to explain this phenomenon [5].

Table 10.2 shows that the use of both internal and external quantitation methods yielded comparable results for sand and clay. When external calibration was employed the same volume of water is added to wet the soil as is analysed in the external standards.

Therefore their will be a small decrease in the volume of the headspace caused by the presence of soil in these vials. It is known that for analytes with high partition coefficients, this small difference in headspace volume may affect the amount extracted [6]. This phenomenon was shown previously in equation 7.6 for the mass balance in a three phase system, where altering the volume of any of the phases will effect the concentration extracted at equilibrium. This effect is even greater in the small systems employed and can lead to errors in quantification. This may also have a small contribution to the results of the water/soil ratio experiments. Therefore the use of standard additions is probably the most valid method of determination since it accounts for the small differences in the headspace volume that will exist between vials when external standards are used to quantify wetted soils.

When comparing the values obtained for extraction of chlorobenzenes from sand by SPME, with the values of Santos et al. [1] (table 10.2), our results are significantly different to those obtained by Santos et. al. and are considerably lower than those stated to be present in the BCR document received. Santos et al. [1] quote the mean Soxhlet values of all the European laboratories which participated in an intercomparison exercise to quantify the levels of chlorobenzenes on the sandy soil. These levels are 0.62 mg/kg for 1,2,3-trichlorobenzene and 1.33 mg/kg for pentachlorobenzene which is also considerably lower than the levels stated by BCR. Our SPME results do not reflect those of Santos et al. or the mean values for the intercomparison exercise.

For the extractions involving Soxhlet, shake flask and ASE (Table 10.4), the RSD's are large for some of these extractions highlighting the lack of repeatability of these techniques under the conditions used. Comparing these results with those acquired by SPME it can be seen that ;

- For clay soil, the manual SPME technique is superior to ASE, shake flask and Soxhlet, probably due to the higher solvent/soil ratio in SPME. However SPME does suffer from high RSD's. The amount of 1,2,4,5-tetrachlorobenzene extracted by ASE with water under harsh conditions (then SPME) is very similar to that of the SPME headspace value.

- With the sandy soil, SPME extracts a higher amount of 1,3,5-trichlorobenzene, 1,2,3-trichlorobenzene and 1,2,4,5-tetrachlorobenzene compared with the other methods. ASE with water followed by SPME gives very low recoveries for all the chlorobenzenes. The extraction of 1,2,4-trichlorobenzene by SPME is comparable to the accelerated solvent extractions with DCM as a solvent. Pentachlorobenzene is exclusively extracted, independent of method, when DCM (and not water) is used as a solvent. Since water is polar and DCM is non-polar, this increase in extraction is undoubtedly due to the non-polar nature of pentachlorobenzene. The recoveries for ASE DCM extractions of pentachlorobenzene increase as the pressure and temperature increase. This is also true for pentachlorobenzene in the clay soil. Comparing the results for extraction of the sandy soil by Soxhlet and SPME the only analyte favourably extracted by Soxhlet is pentachlorobenzene. All the other chlorobenzenes were favourably extracted by SPME.

It is interesting to compare the recoveries of chlorobenzenes by simple shake flask extractions with Soxhlet. It can be seen that for sandy soil shake flask is superior to Soxhlet and for clay soil the results are comparable. This suggests that when DCM is used as a solvent, the effect of heat does not facilitate analyte release from the matrix. This is also true when comparing the ASE DCM extractions of clay with shake flask.

Although the reference materials contained high amounts of chlorophenols, the chromatograms for SPME headspace of sandy and clay soils did not indicate the extraction of these analytes. This selectivity is an advantage to the sensitivity of the technique developed. The only other major compounds extracted by headspace SPME were the isomers of lindane, which eluted in a later part of the chromatogram to chlorobenzenes (figure 8.7-8.8).

The only major problem in quantification was of the ASE water extracts. These were heavily contaminated with dichlorophenols and trichlorophenols which interfered with the chromatography of the chlorobenzenes.

10.3.2 PAH's

Typical chromatograms for the separation of the PAH's extracted from the reference material, contaminated soils, garden soil and exhaust soot, using the PDMS fibre, have been shown previously (figs. 8.9-8.14).

10.3.2.1 Quantitation of PAH's in a reference material

The results of extractions of PAH's from LGC 6138 standard reference material are given in table 10.5 and the RSD's are in brackets next to the average. The certified values are also given for comparison.

Table 10.5

Extraction of PAH's from high carbon CRM, LGC 6138 soil by SPME with a PDMS fibre

	Coal-carbonisation site soil (CRM, LGC 6138)		
	Certified values mg/kg	**0.003 g/0.15 ml water** **Average** mg/kg n = 5 (% RSD)	**1 g/1 ml water** **Average** mg/kg n = 5 (% RSD)
Naphthalene	**32**	**24.55** (5.3)	**13.30** (8.15)
1-methylnaphthalene		**7.14** (10.93)	**3.54** (12.86)
Fluorene	**15.3**	**7.44** (3.8)	**1.25** (10.36)
Phenanthrene	**114**	**77.38** (2.69)	**5.53** (13.28)
Anthracene	**22**	**6.95** (2.36)	**1.32** (10.96)
Fluoranthene	**118**	**37.00** (14.43)	**1.3** (14.28)
Pyrene	**103**	**22.10** (15.06)	**0.81** (13.49)

The first observation from the results in table 10.5 is that 100 % recovery of any of the PAH's is not achieved. It is also clear that when a larger sample is used, the soil/water ratio is possibly causing lower recoveries. The recoveries when using a lower mass are much higher due to the amount of the solvent per unit mass being greater. A further explanation is that which involves the size of the extraction vial. Since the effects of equation 7.6 cause equilibration to alter depending on the volume of each phase present, this will have a

considerable effect on the extraction of PAH's since the volume of the headspace is so large. The recoveries tend to be lower for PAH's with higher Kow and Henry's constants, which suggest strong interactions with the high carbon content of the reference material.

Although the recoveries were generally low, SPME overcame the problem of sulphur interference with no pre-requisite of sample cleanup which is generally a lengthy process. It may be that the recoveries may be improved and the same selectivity observed (concerning sulphur) by modifying the SPME method by adding a small amount of DCM (0.15 ml) and analysing the headspace.

10.3.2.2 Quantitation of PAH's in environmental matrices

Table 10.6 on the following page shows the levels of PAH's extracted an quantified by this method on the contaminated soils, exhaust soot and the 'uncontaminated' garden soil. As extractions were carried out in duplicate, these results are in brackets next to the average value.

Table 10.6

PAH's extracted and quantified (mg/kg) from various solid matrices, by SPME headspace at 80 °C

	Contaminated Exhaust soot	Contaminated Soil 1	Contaminated Soil 2	Contaminated Soil 3	Uncontaminated Garden soil
	Average mg/kg n = 2 (results)	Average mg/kg n = 2 (results)	Average mg/kg n = 2 (results)	Average mg/kg n = 2 (results)	Average µg/kg n = 2 (results)
Naphthalene	**0.65** (0.68, 0.63)	**23.61** (23.93, 23.31)	**0.19** (0.17, 0.21)	**0.32** (0.32, 0.32)	**9.7** (8.3, 11.1)
1-methylnaphthalene	**0.13** (0.14, 0.12)	**9.78** (9.4, 10.17)	**0.029** (0.027, 0.031)	**0.23** (0.23, 0.23)	**1.5** (1.5, 1.6)
Fluorene	**0.24** (0.26, 0.22)	**19.77** (17.4, 22.15)	**0.045** (0.043, 0.046)		
Phenanthrene	**1.20** (1.23, 1.17)	**60.82** (51.6, 70.6)	**0.17** (0.17, 0.17)	**0.078** (0.078, 0.079)	**2.0** (2.0, 2.0)
Anthracene	**0.24** (0.25, 0.23)	**6.48** (5.52, 7.45)	**0.020** (0.019, 0.022)	**0.026** (0.027, 0.025)	**0.46** (0.33, 0.59)
Fluoranthene	**0.28** (0.28, 0.27)	**16.28** (12.89, 19.69)	**0.034** (0.039, 0.03)	**0.026** (0.026, 0.027)	**1.4** (1.7, 1.2)
Pyrene	**0.16** (0.16, 0.15)	**9.12** (7.58, 10.66)	**0.019** (0.022, 0.016)	**0.021** (0.02, 0.022)	**0.86** (0.84, 0.89)

The results in table 10.6 show a range of different levels of contamination on the soils, for the PAH's quantified. For example 0.0097 and 23.61 mg/kg of naphthalene on garden soil and 'contaminated site 1' respectively. As expected, garden soil has very low levels of PAH's in comparison with the other soils.

The water/soil ratios for maximum extraction of the PAH contaminated soils were not optimised, therefore (especially with the heavily contaminated soils) it is likely that we are observing an underestimation of the actual levels present. These soils were taken from industrial sites and from urban vehicles. Leaching of PAH's into water supplies from the heavily contaminated industrial sites and direct inhalation of the exhaust particles in the urban environment are two worrying consequences of pollution.

The analysis of diesel exhaust soot showed a high concentration of PAH's which on finer particles may be easily inhaled. PAH's extracted by Soxhlet from a PM_{10} sample filtered from urban air have been found to have levels of 0.29 mg/kg fluorene, 4.9 mg/kg phenanthrene, 0.36 mg/kg anthracene, 8 mg/kg fluoranthene and 6.9 mg/kg pyrene [7]. Heavier PAH's including Benz[a]anthracene, Crysene, Benzo[k]fluoranthene, Benzo[a]pyrene and perylene were also present in similar concentrations. These levels are slightly higher than those found in our particles. The reason for this, apart from different sampling location, will be due to specific binding of PAH's to different size fractions (i.e. airborne v's heavier soot particles) and also on the extraction conditions used to quantify these levels. As observed with our results for the PAH reference material there is a trend for

increased binding (decreased analyte release) as the Kow and Henry's constants increase. The levels of the heavier PAH's on these soils may therefore be higher.

The sample method developed for chlorobenzenes was used for PAH's. Only the volatile and semivolatile PAH's could be analysed under the SPME and GC parameters employed. The tobacco (chapter 8.0), soils and particles analysed by this method may contain heavier much less volatile PAH's. Non volatile PAH's may be extracted at high temperatures by SPME if a small volume of non-polar solvent was used instead of water. These compounds could be eluted and quantified if a more polar column and a higher final oven temperature was used (i.e. 300 °C).

Based on our results it is important to note that chlorobenzenes and PAH's which are bound to solid matrices in the environment may possess hazards to health when they are in contact with water. As it has been shown, there is a higher concentration present in the headspace when water is added to the soil. Therefore, this would further increase the risk of exposure due to direct inhalation. For example, particles with PAH's attached are known to accumulate in the alveoli of the lungs where they encounter a moist environment with a large headspace at body temperature (c.a. 35 °C). It has been shown that adding water to the sample facilitates release of analytes from the matrix. At body temperature more volatile PAH's will be mainly released from particles. PAH's with high Henry's constants (naphthalene and 1-methylnaphthalene) may be excreted from the lungs via respiration, shown by loss from solution in a stirred system (mimicking breathing). Whilst if heavier PAH's are released from the particles (fluorene, phenanthrene, anthracene, fluoranthene and

pyrene) they will prefer to stay in solution and partition into the wall of the alveoli or through the wall and directly into the blood. Much heavier PAH's will have very slow partitioning into water due to their high Kow's and once released there may be a size hindrance from crossing the alveoli membrane. However they may become attached to proteins in the membrane, directly effecting the lung cell lining.

10.4 Summary and conclusions

Solid phase microextraction (SPME) was chosen to analyse solid matrices by headspace as it has been shown to be an excellent tool for the extraction, preconcentration and identification of low levels of analytes from a diverse range of matrices without the requiring the use of organic solvents which is so often the case in extraction methodologies.

A method was developed using SPME to extract analytes from the static headspace above heated contaminated soils. The method was applied to the quantification of these analytes using external and internal calibration. With modifications, these methods of quantitation are equally applicable to any solid matrix.

Headspace analysis was found to remove interferences known to be present in the reference soils whilst at the same time, preconcentrating the analytes of interest.

The conditions optimised in this study were used to maximise extraction by minimising the affinity of analytes towards their host matrix. The use of headspace SPME to

analyse water standards may overcome the problem of quantifying the levels of analytes on solid samples.

The use of Celite as a standard was found to give unpredictable results whilst water standards showed excellent linearity.

The results presented for SPME, ASE, Soxhlet and shake flask extraction, suggest that only a fraction of the chlorobenzenes and PAH's have been extracted under the conditions used. However, the levels of chlorobenzenes and PAH's on the matrices analysed may become increasingly bound with time, as geometrical orientations are reached of lowest energy.

Since full analyte release under the conditions used in these experiments is not achieved, then it is unlikely to be achieved under environmental conditions. Therefore the results presented may actually be higher than those which are likely to be available for 'short term' exposure to plants and animals.

The discrepancies in the results by SPME mirrored by the high RSD's, reflect the difficulty in reproducing all the conditions in the manual system. Therefore, the overall accuracy/presision of this method relies heavily on the analyst. Quantification using SPME should be possible if the extraction conditions can be 'fully' optimised and the overall method is reproducible. This would eventually allow SPME to be used not only as a technique for matrix screening and quantifying liquid samples, but also as an attractive

alternative to traditional solid extraction/quantification methods. Advances in automation of this type of technique would be of great benefit in the quantitation of pollutants in solid matrices. This is imperative if the technology is to be applied for routine analysis. Heating and/or stirring will need to be integrated into the equipment in future to be able to diversify applications.

Knowledge of the bioconcentration kinetics of chemicals in aqueous organisms is important in order to predict the risk of exposure of organisms to the various pollutants in the aquatic environment. For this reason the US Environmental Protection Agency recently published a series of standard biological assays for assessing toxicity and bioaccumulation of contaminants from sediments [8]. If K_{SPME} can be related to Kow and bioconcentration factors then it may become a useful method for measuring and predicting bioconcentration as well as partitioning in soils. It will also be more rapid than present methods of extraction and analysis.

At this stage in method development SPME can certainly be applied to qualitative screening and to semi-quantify levels of pollutants. One of the most important factors found with determining chlorobenzenes was the water/soil ratio as the volume of solvent increased so did the amount extracted. Larger masses and water/soil ratios could be used in conjunction with automated analysis to improve method repeatability, accuracy and sensitivity. With its potential to monitor all forms of matrices coupled with the advantages listed in chapter 2.0, SPME will undoubtedly become a prevalent analysis tool in many future applications.

10.5 Novel applications of SPME

With the growing awareness of the simplicity of the SPME technique, many novel applications have recently been developed and its use has even appeared in the RSC publication "Chemistry in Britain" [9]. SPME has been applied to extraction of the peptide leucine-enkephalin, and ferrocene [10], volatiles from headspace of the urine of traffic fatality victims [11], amphetamines in urine [12], insect epicuticular hydrocarbons from social wasps [13], steroids from human serum [14] and flavour and fragrance analysis [15].

Forsyth and Dusseault [16] used SPME to quantify the accumulation of a gasoline antiknock additive in milk and spring water exposed to gasoline vapours. Values were given for the oral lethal dose in rats of this additive, and the levels they detected in milk and water. Interestingly, from their values, one can observe that for a person to reach a lethal dose of this additive (assuming he or she weighs 80 kg) they would have to drink contaminated milk non stop for over 3730 years (or 176,000 years for water).

In future applications SPME could be used by forensic, medical and pharmaceutical companies to monitor pathways of chemicals in the body through saliva gastric fluid, tissue samples, blood, urine and faeces to rapidly piece together a comprehensive picture of a chemicals disappearance, metabolism, bioconcentration and secretion.

10.6 References

1. F.J. Santos, M.N. Sarrion and M.T. Galceran, *J.Chromatogr. A,* **771** (1997) 181.
2. H.J. Vandenburg, A.A. Clifford, K.D. Bartle and S.A. Zhu, *Anal. Chem.*, **70** (1998) 1943.
3. W. Karcher, G. Kuhnt, M. Herrmann and H. Muntau, Proceedings of the 5th International workshop Environmental behaviour of Pesticides and Regulatory Aspects Brussels (1994) p112, April 26-29, Edited by A. Copin, G. Houins, L. Pussemier, J.F. Salembier.
4. J.C. Miller and J.N. Miller, In *'Statistics for Analytical Chemistry, 3rd Edition'*; Ellis Horwood Ltd., Cooper street, Chichester, West Sussex, UK, (1993).
5. K.D. Bartle, A.A. Clifford, S.B. Hawthorn, J.J. Langenfield, D.J. Miller and R.J. Robinson, *Supercrit. Fluids,* **3** (1990) 143.
6. T. Gorecki and J. Pawliszyn, *Analyst,* **122** (1997) 1079.
7. J.H. Clemons, L.M. Allan, C.H. Marvin, Z. Wu, B.E. McCarry, D.W. Bryant and T.R. Zacharewski, *Environ. Sci. Technol.*, **32** (1998) 1853.
8. US Environmental Protection Agency, Methods for measuring the toxicity and bioaccumulation of sediment associated contaminants with freshwater invertabrates, EPA/600/R-94/024. Environmental Protection Agency , Washington DC (1994)
9. D. Bradley, 'The Royal Society fo Chemisty', *Chemistry in Britain*, p17 July (1998).

10. M.E. Cisper, W.L. Earl, N.S. Nogar and P.H. Hemberger, *Anal. Chem.*, **66** (1994) 1897.

11. W.E. Brewer, R.C. Galipo, S.L. Morgan and K.H. Habben, *J. Anal. Toxicol.*, **21** (1997) 286.

12. SPME. Supelco Application notes: **58** and **83** (1994).

13. G. Moneti, F.R. Dani, G. Pieeraccini and S. Turillazzi, *Rapid Communications in mass spectrometry*, **11** (1997) 857.

14. P. Okeyo, S.M. Rentz and N.H. Snow, *J. High Resolut. Chromatogr.*, **20** (1997) 171.

15. SPME Supelco Bulletin: **869** (1995) 1.

16. D.S. Forsyth and L. Dusseault, *Food Addit. Contam.*, **14** (1997) 301.

Chapter 11.0

Achievement of aims and ideas for future work

11.1 Achievement of aims

Soils were collected from varying locations, and their properties characterised to enable correlations to be made between soil properties and the fate of phenols applied to soil. One of the least facile aims of this investigation was that of appreciating the complexity of soil, and this required the greatest patience.

Methods were developed to extract organic pollutants from environmental samples. Shake flask extraction and SPME have combined low cost, minimal toxic solvent usage, simplicity and low sample preparation times. These methods provide viable alternatives to traditional sample preparation techniques which are time consuming and use large quantities of toxic solvents.

- **Part A: The fate of phenolic compounds in the environment**

Shake flask coupled with HPLC has been shown capable of charting the disappearance of phenols with time in artificially spiked soil, and may be used to routinely monitor soil for phenols or other contaminants. All the soils collected were analysed for their sorption potential towards phenols with time since application. 1-naphthol was found to be lost from the majority of the soils and degradation products were found to emerge with time. Relating the sorption of phenols to the soil properties characterised was fruitless. It may be that uncharacterised properties contribute toward sorption along with characterised properties.

After method development, the sorption behaviour of phenol and 1-naphthol on collected soils was found by batch equilibration partitioning experiments. These experiments were used to monitor the sorption behaviour of phenol and 1-naphthol in soils collected from various locations. This is an important method, as it enables predictions to be made about the fate of pollutants in the environment based on correlations made between the degree of sorption and the properties of the soil.

Although the partition coefficients can be used to predict the impact of these chemicals on the different soils studied in real environmental situations the partitioning/sorption could not be adequately related to the measured soil properties. This poses limitations when predicting sorption by relating it to soil properties.

- **Part B: Determination of organic pollutants in environmental matrices using solid phase microextraction (SPME)**

The SPME methods developed in this project have shown its capability to analyse both aqueous and solid samples to a high degree of sensitivity. This is due to its ability to extract and pre-concentrate analytes in one step, coupled with the performance of the detector employed. A method has been successfully developed to selectively 'extract', 'separate', 'identify' and 'semi-quantify' chlorobenzenes and PAH's from liquid and solid matrices using SPME headspace coupled with gas chromatography and mass spectral detection. Detection was achieved for PAH's on uncontaminated garden soil at levels of nmoles/kg.

This method preconcentrates analytes and doesn't involve sample cleanup or any organic solvent. When automation was employed to analyse liquid samples, SPME was able to analyse nmole/dm^3 levels of chlorobenzenes with high sample throughput and repeatability. The use of sample agitation was found to increase the extraction rate of PAH's thus improving sensitivity in a minimum amount of time.

11.2 Ideas for future work

- **Part A: The fate of phenolic compounds in the environment**

Further soil characterisation studies are required to relate the results of the experiments involving the sorption/partitioning of phenols, to soil properties. The type and amount of clay present in the soils should be determined as it may be a key factor in determining sorption. The identification of degradation products of phenols could also be investigated.

Organic matter was not found to be a sole contributing factor to sorption, however may contribute alongside other soil properties. The removal of organic matter from the soils may be achieved by muffle heating the soils at 800 °C. These soils could then be spiked with the phenols and analysed over time to evaluate the importance of organic content. The humic acids could also be extracted from each soil based on a method proposed by Garbarini [1] and the soil matrix spiked with phenols. This would enable the assessment of the individual contribution to sorption/partitioning of humic acids. Alternatively, the inorganic carbonate

could be removed from each soil by treating with acid and the soil then spiked. Either of these techniques will provide information regarding the roles of organics and inorganics in the sorption process in soil.

SPME coupled with HPLC could be used to analyse low concentrations of chemicals in soil/water systems. This would provide a much simpler method than the batch equilibration systems described in chapter 6.0. SPME headspace analysis could eliminate problems in quantification related to dissolved humic acid bound contaminants.

- **Part B: Determination of organic pollutants in environmental matrices using solid phase microextraction (SPME)**

Real environmental water samples should be obtained to evaluate the method developed for analysis of chlorobenzenes and PAH's from aqueous samples. Further optimisation of this technique may be achieved to lower equilibration times by agitation.

Further method development is required to quantify analytes on solid matrices. This should include research into the water/soil ratio for individual soils and perhaps the application of a small amount of non-polar solvent to aid extraction of nonpolar analytes.

Once these two methods are fully optimised and found to produce repeatable results (lower RSD's) the analysis and quantification of pollutants from various matrices could be evaluated. Matrices could include milk or egg yolks. As cows milk contains low levels of fat

one can assume this is an ideal medium for bioconcentration of organic chemicals such as pesticides on grass. Chicken egg yolks are a source of protein and may also be a medium for accumulation of toxins. One could follow the fate of toxins from contaminated chicken feed through to egg yolks.

It may also become possible in future applications to chemically bond fibers with thermally stable catalysts and use the fibres coupled with GC-MS to monitor rates of chemical conversions.

11.3 References

1. D.R. Garbarini and L.W. Lion, *Environ. Sci. Technol.*, **20** (1986) 1263.

Appendix A:

Shake flask extraction of phenols from aged soils

This appendix gives further results from chapter 5.0 for the influence of soil sorption time on the recoveries/chromatography of phenols extracted from soil by shake flask. The 'key' below identifies the phenols in the results on the following pages.

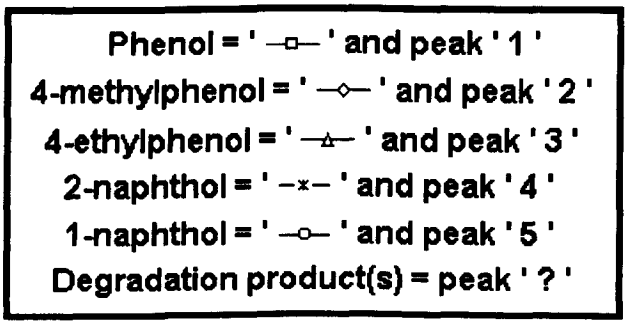
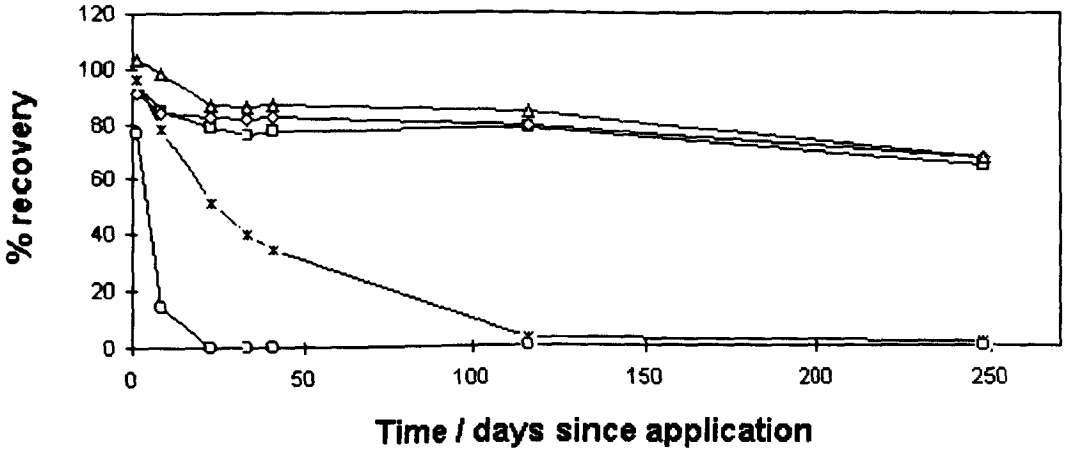


Figure A1

Influence of soil sorption time on the recoveries of phenols extracted from site 2 BCg (loamy sand) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 2 BCg (loamy sand) by shake flask.

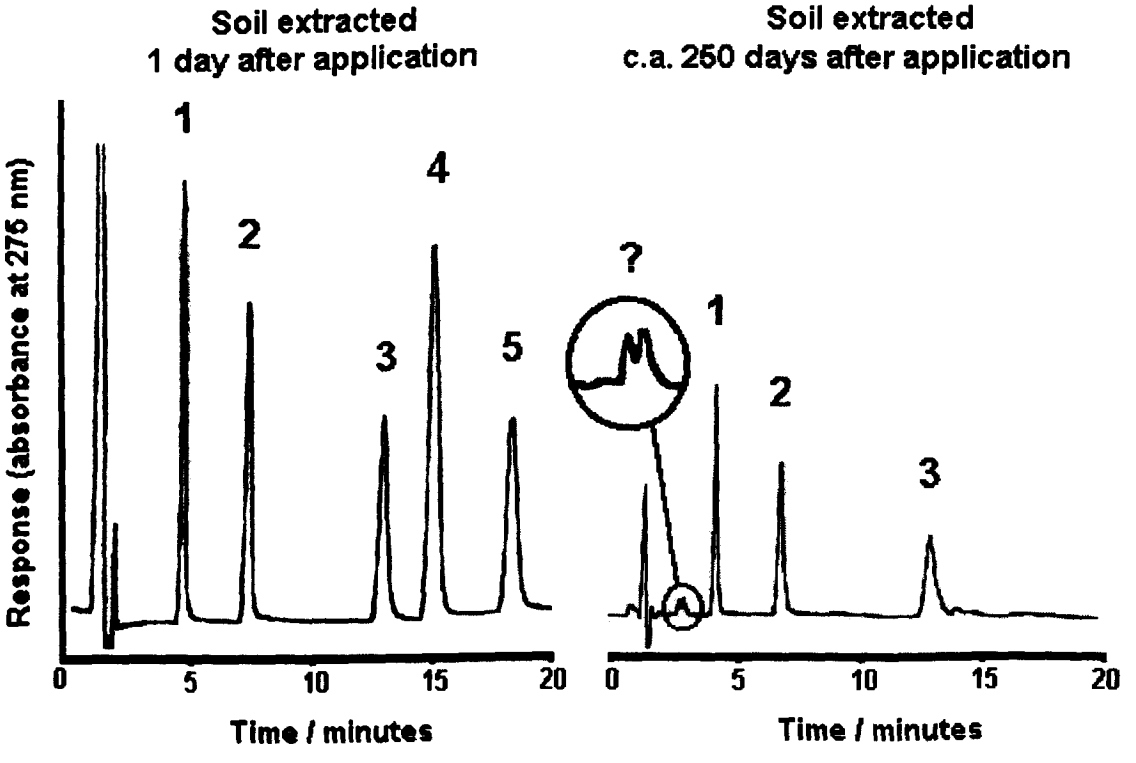
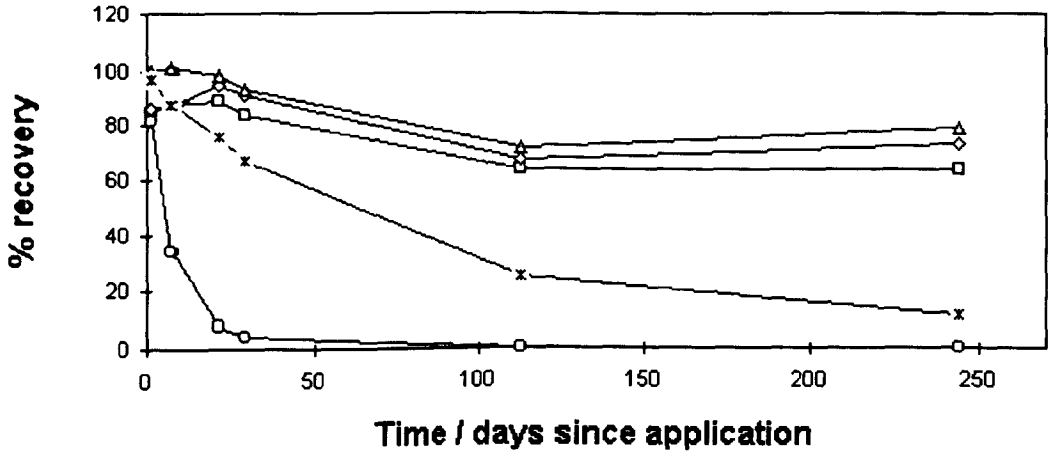


Figure A2

Influence of soil sorption time on the recoveries of phenols extracted from site 1 BhS (humus/sesquioxide) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 1 BhS (humus/sesquioxide) by shake flask.

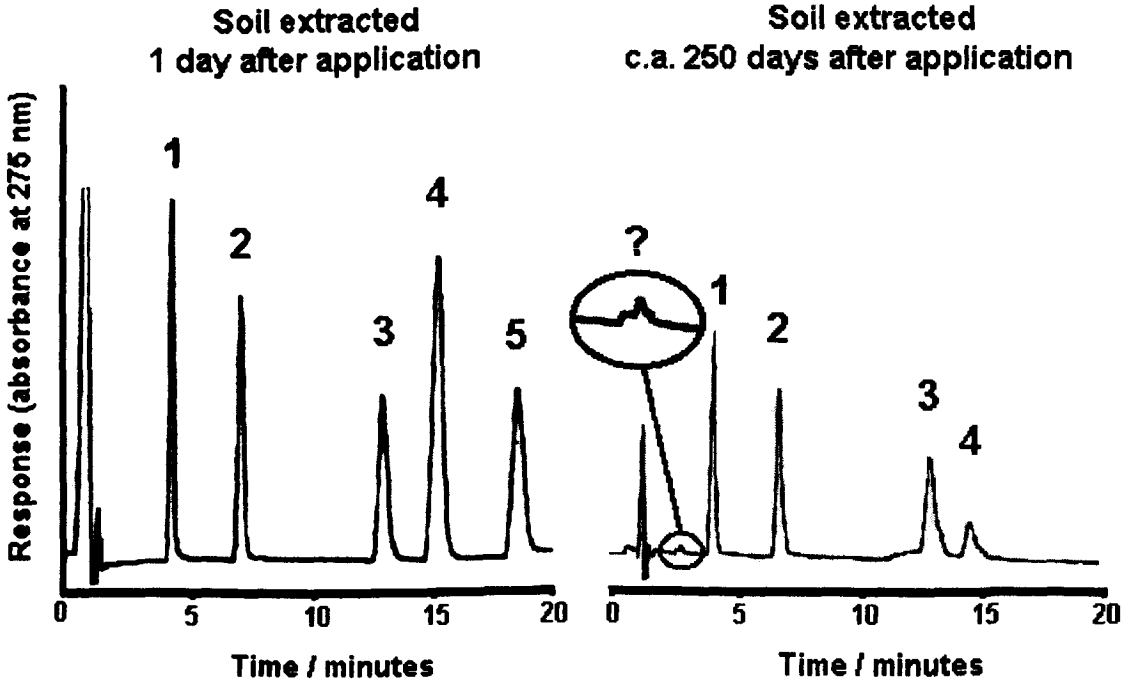
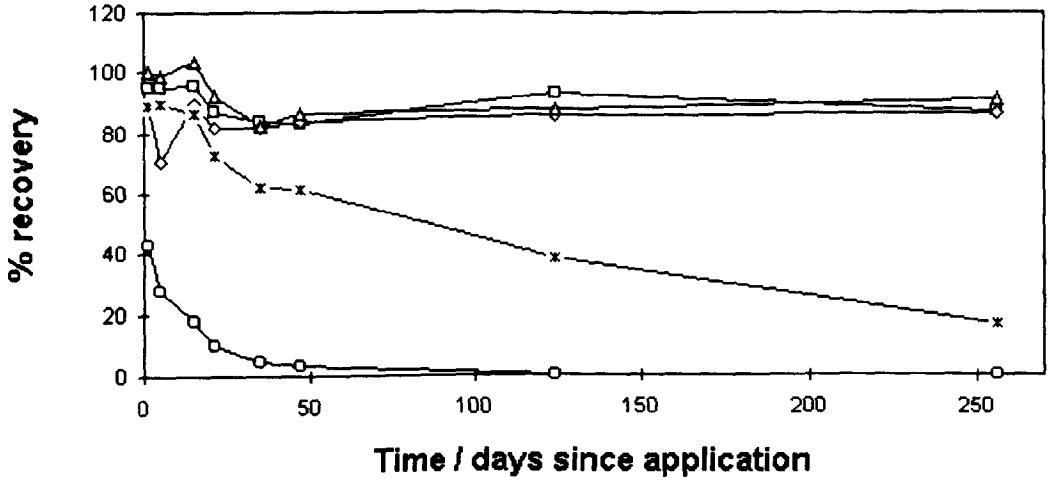


Figure A3

Influence of soil sorption time on the recoveries of phenols extracted from garden soil by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site garden soil by shake flask.

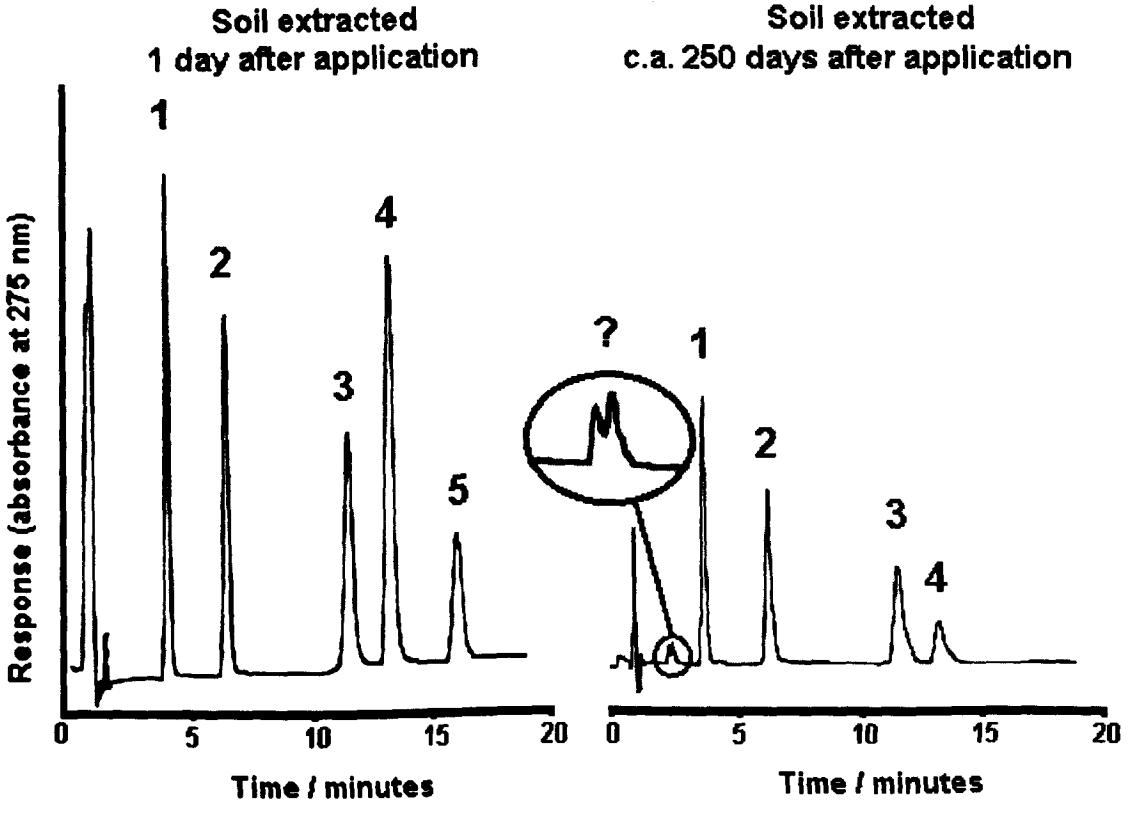
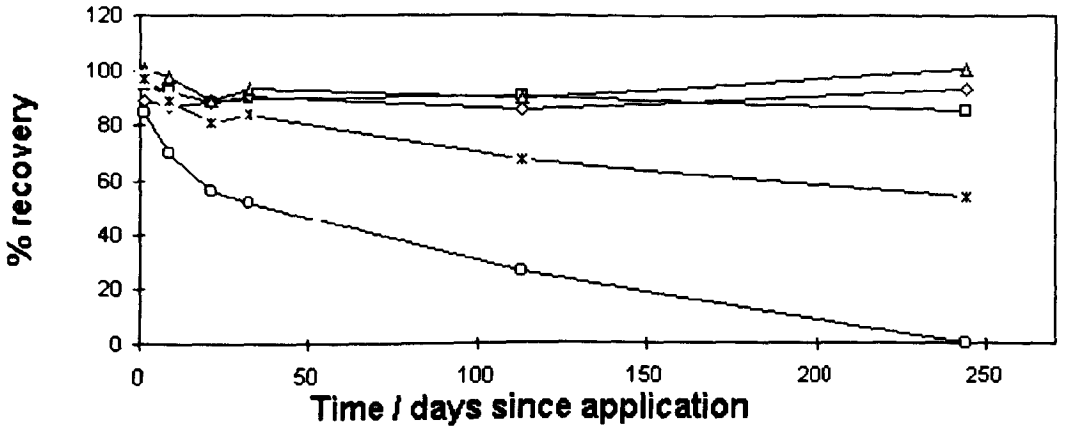


Figure A4

Influence of soil sorption time on the recoveries of phenols extracted from site 2 Ah (humus rich + minerals) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 2 Ah (humus rich + minerals) by shake flask.

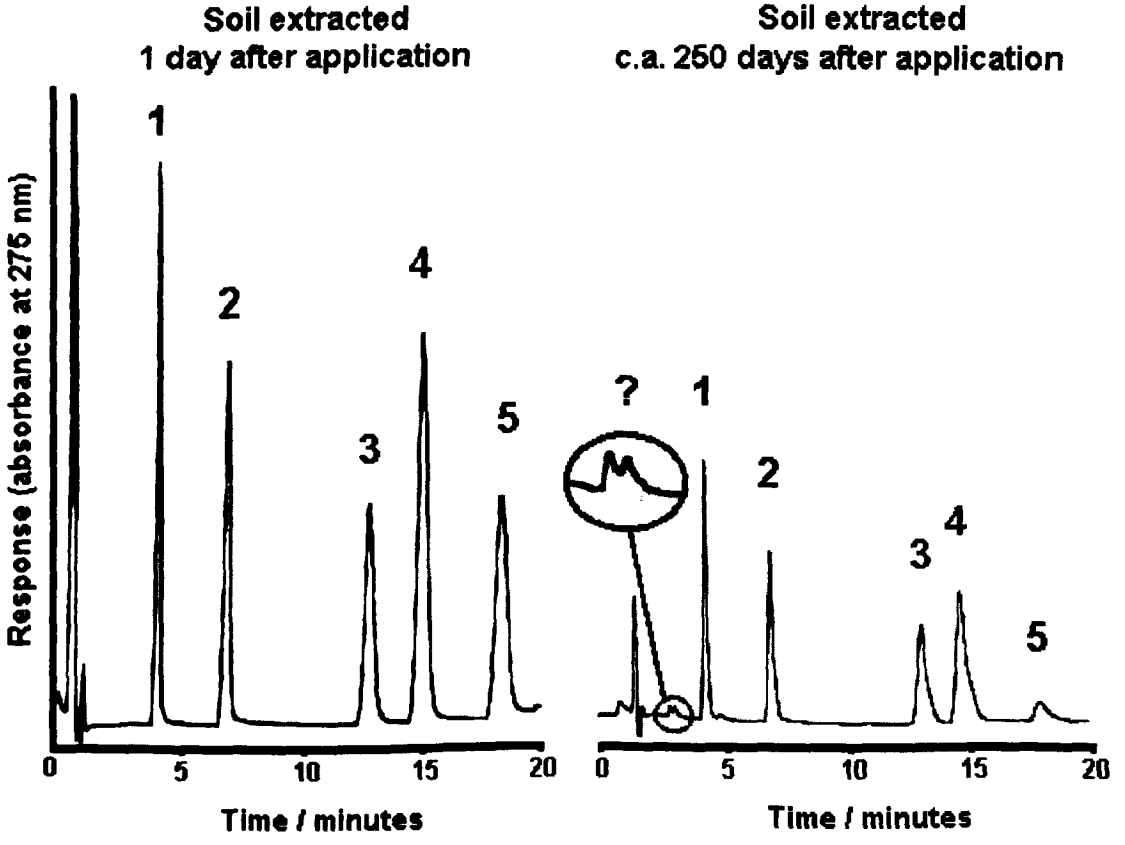
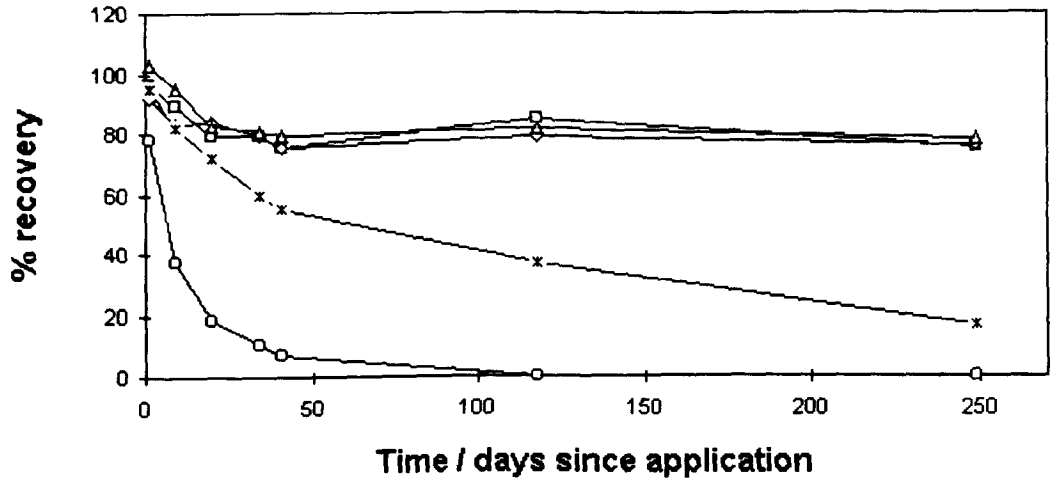


Figure A5

Influence of soil sorption time on the recoveries of phenols extracted from site 2 Bg (loamy sand) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 2 Bg (loamy sand) by shake flask.

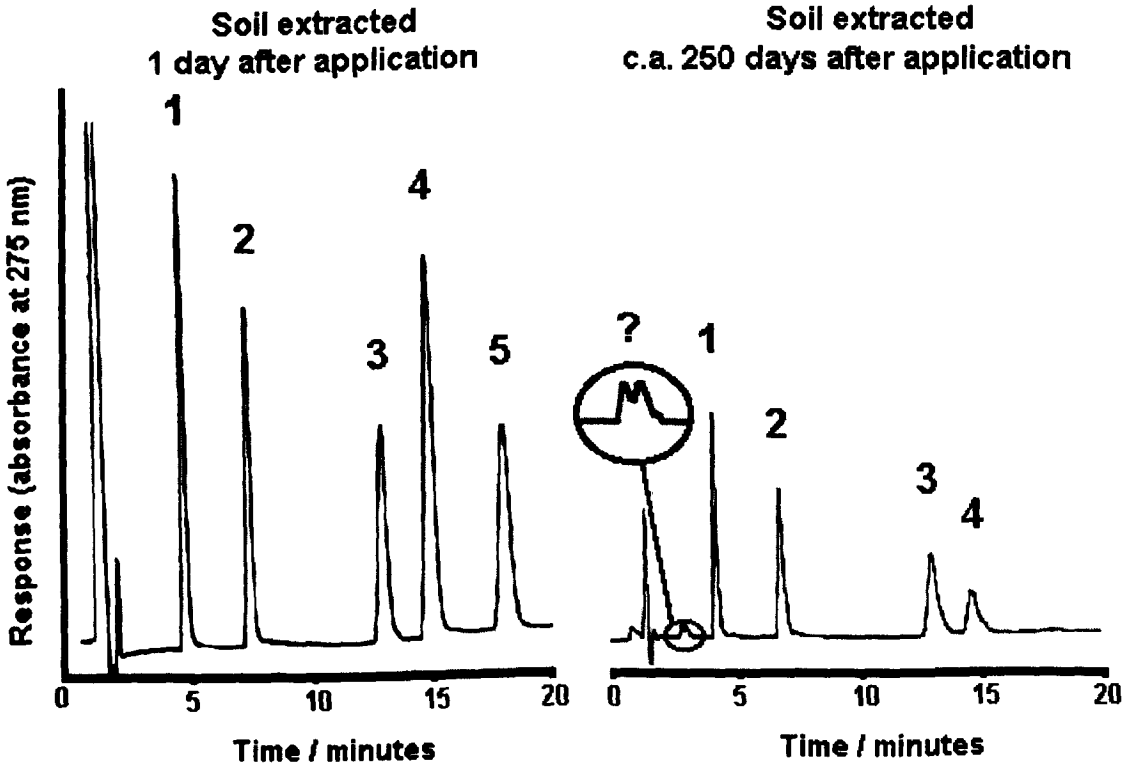
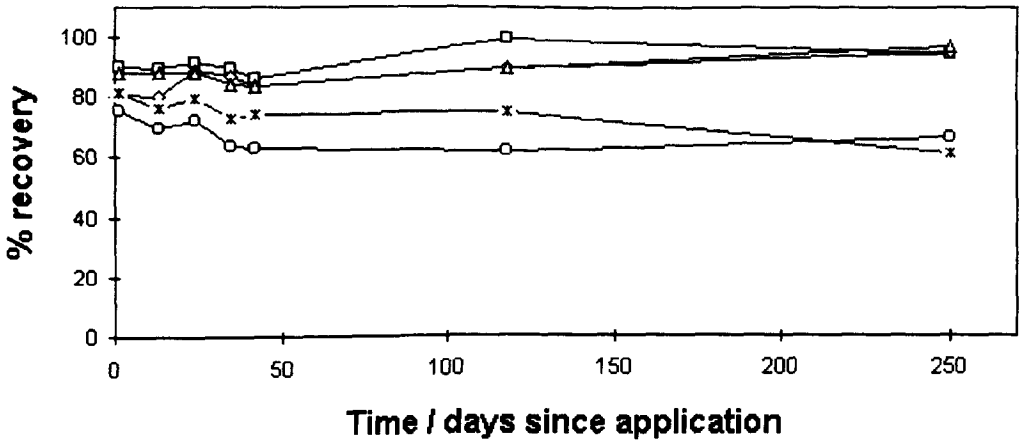


Figure A6

Influence of soil sorption time on the recoveries of phenols extracted from site 3 01 (fibrous peat) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 3 01 (fibrous peat) by shake flask.

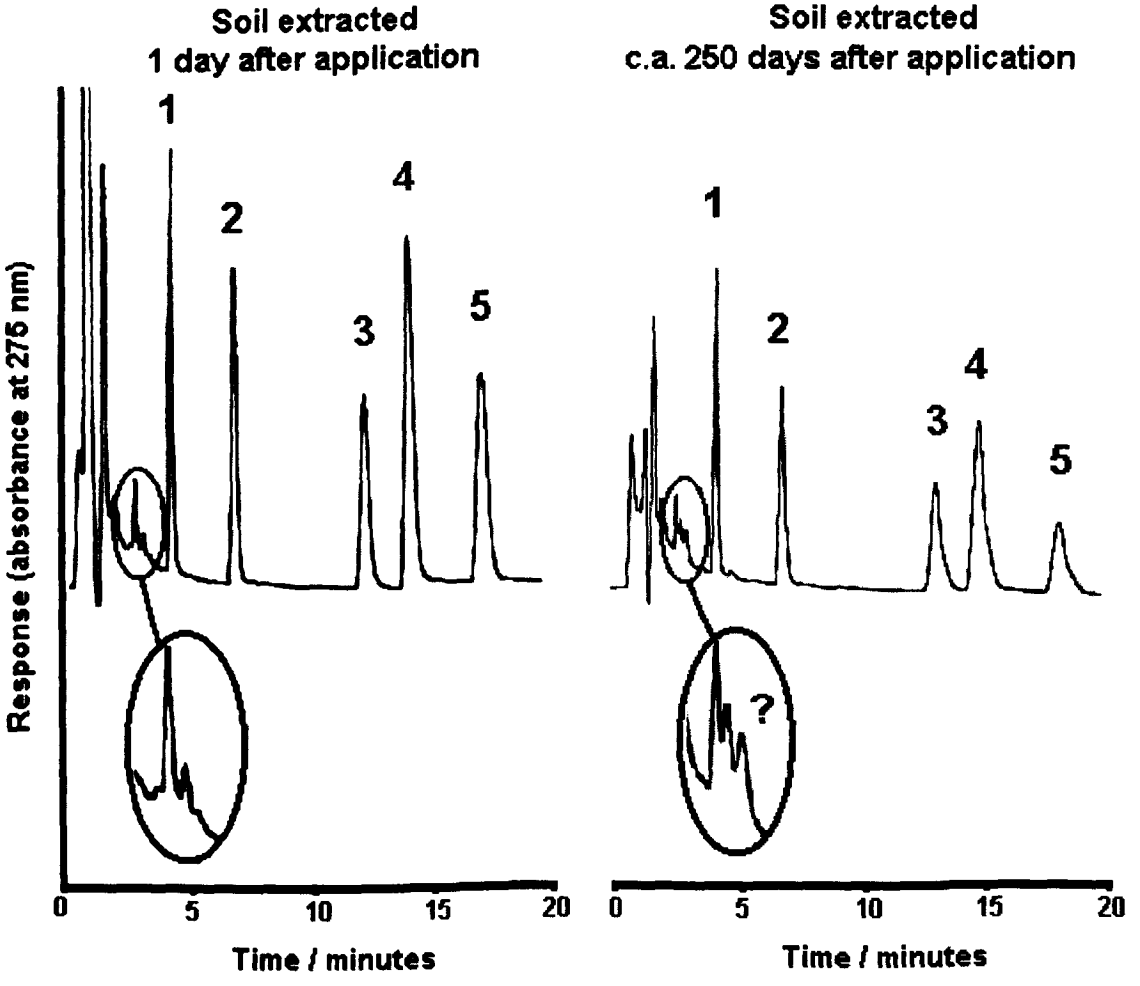
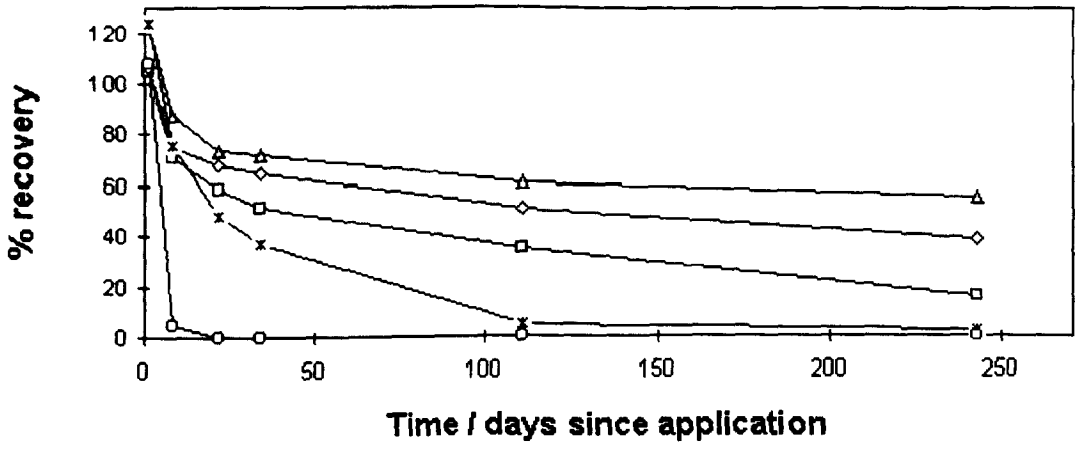


Figure A7

Influence of soil sorption time on the recoveries of phenols extracted from sand by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from sand by shake flask.

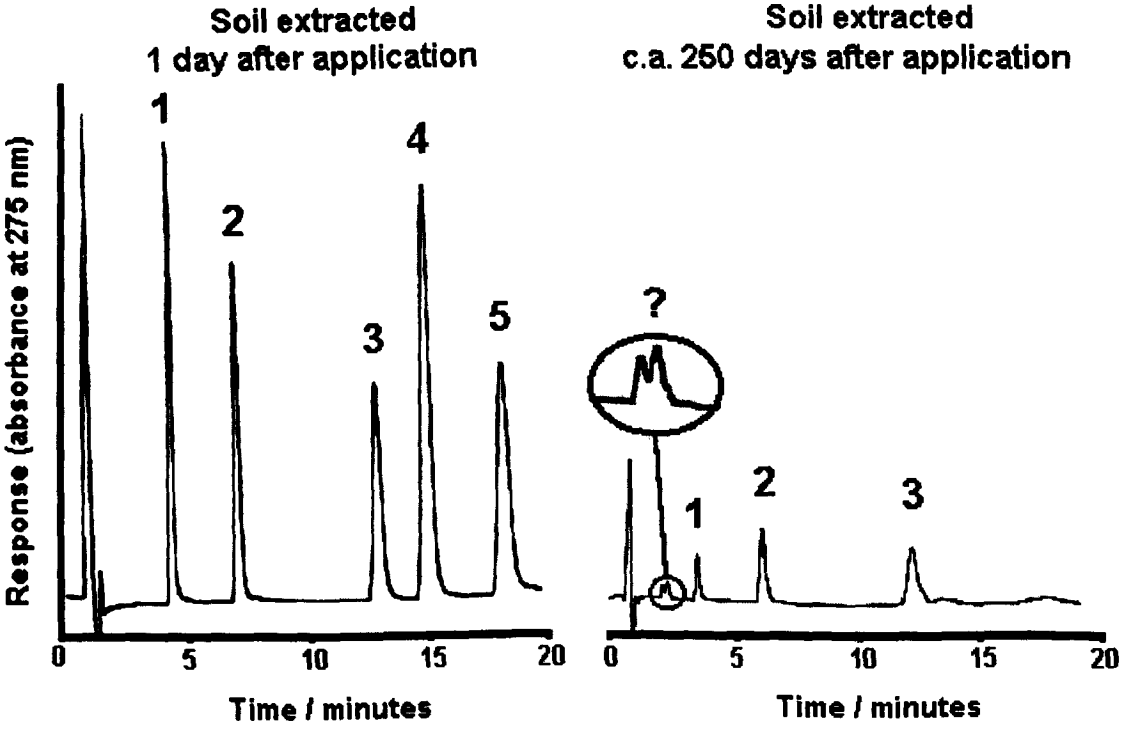
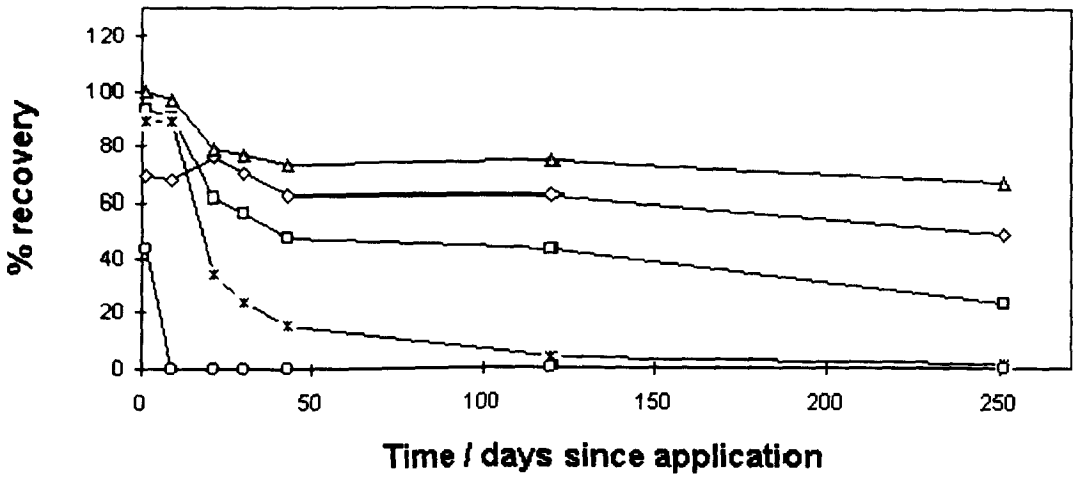
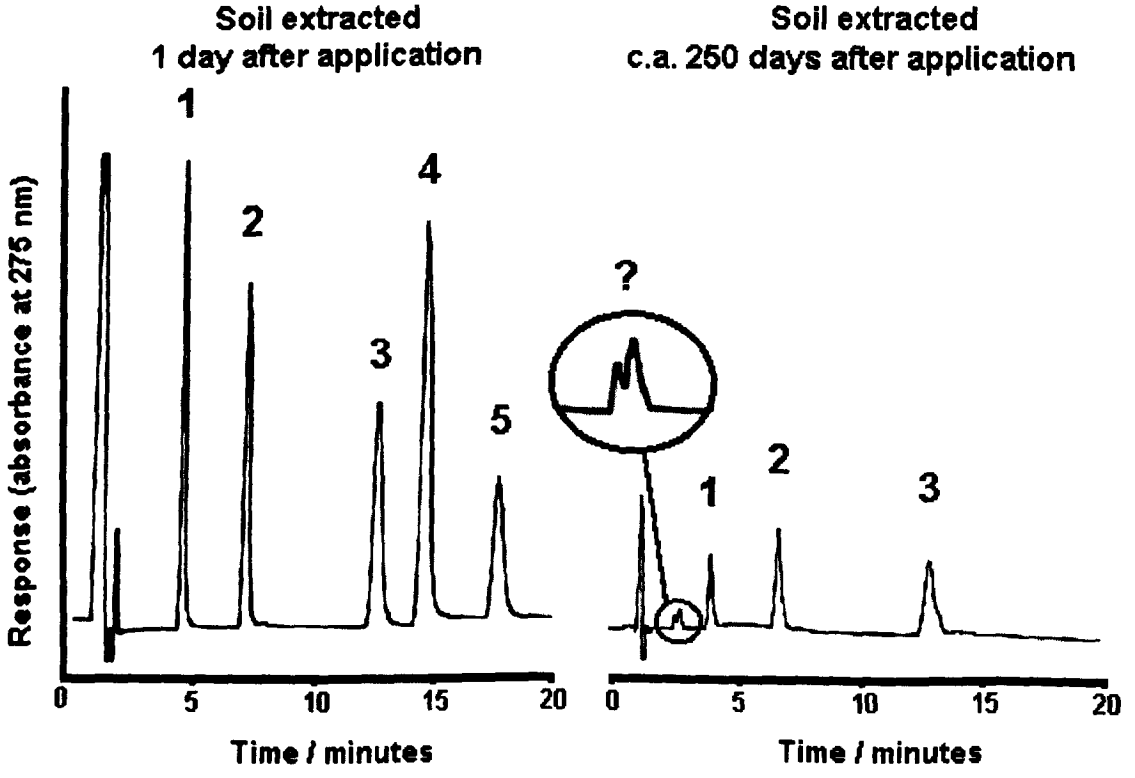


Figure A8

Influence of soil sorption time on the recoveries of phenols extracted from site 4 R (limestone/sandy) by shake flask.



Influence of soil sorption time on the chromatography of phenols extracted from site 4 R (limestone/sandy) by shake flask.



Appendix B:

Partitioning of radiolabelled phenol and 1-naphthol in soil

This appendix gives further results from chapter 6.0 for the influence soil type on the sorption/desorption isotherms of phenol and 1-naphthol measured by batch equilibration. The 'key' below identifies the isotherm type in the figures on the following pages.

▪ Sorption isotherm ○ Desorption isotherm

Figure B1

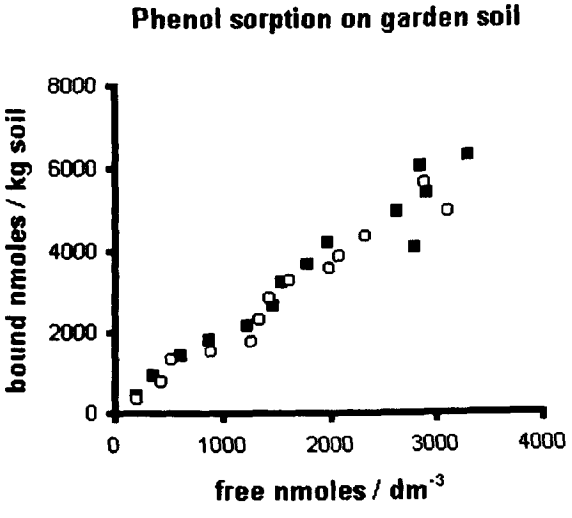


Figure B2

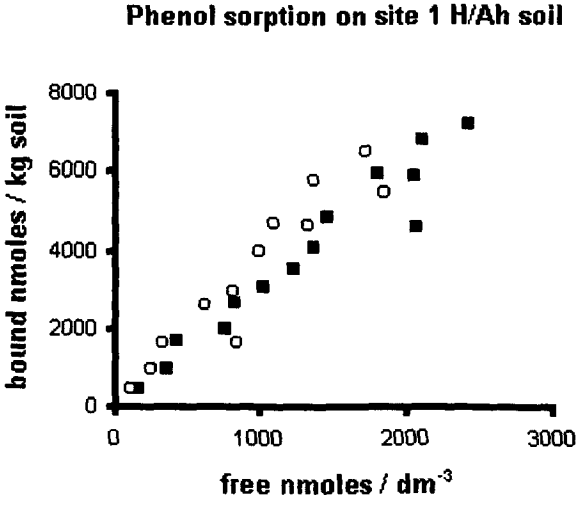


Figure B3

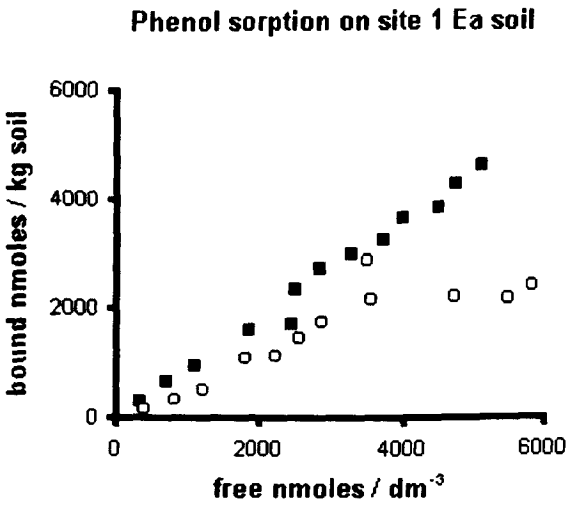


Figure B4

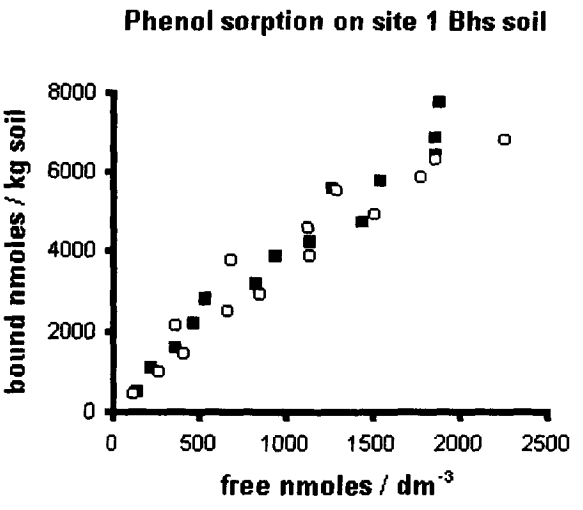


Figure B7

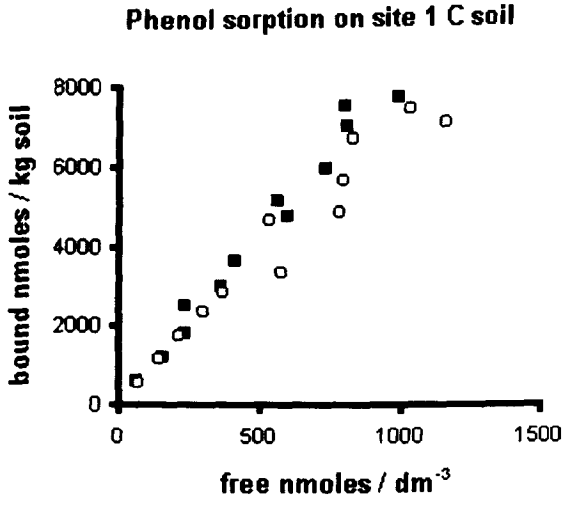


Figure B6

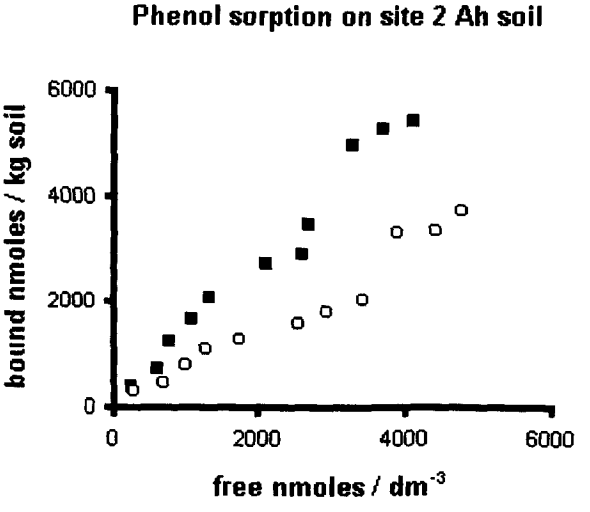


Figure B7

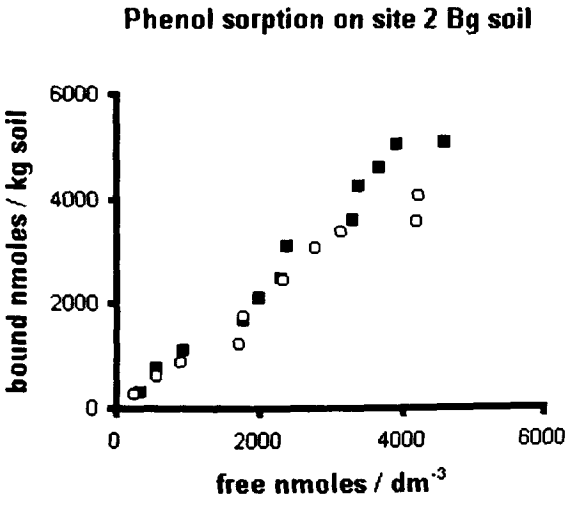


Figure B8

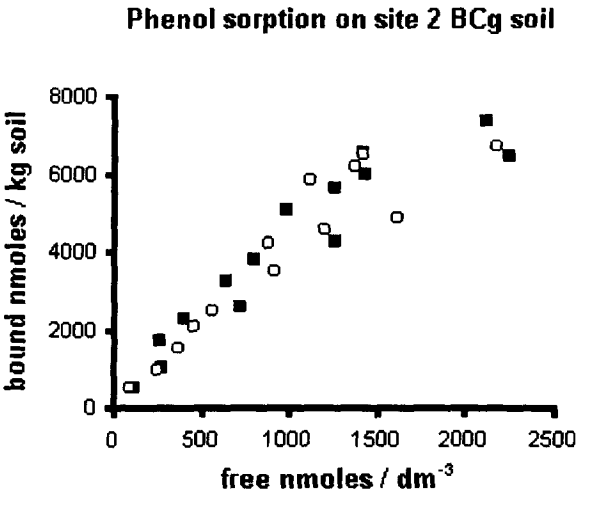
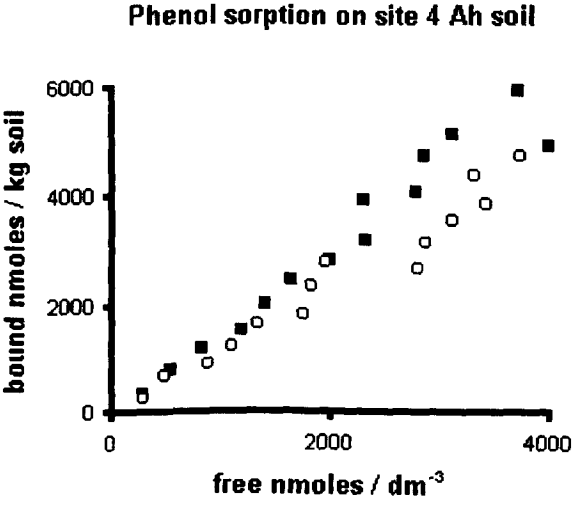


Figure B9



Sorption and desorption isotherms for 1-naphthol on soils

■ Sorption isotherm ○ Desorption isotherm

Figure B10

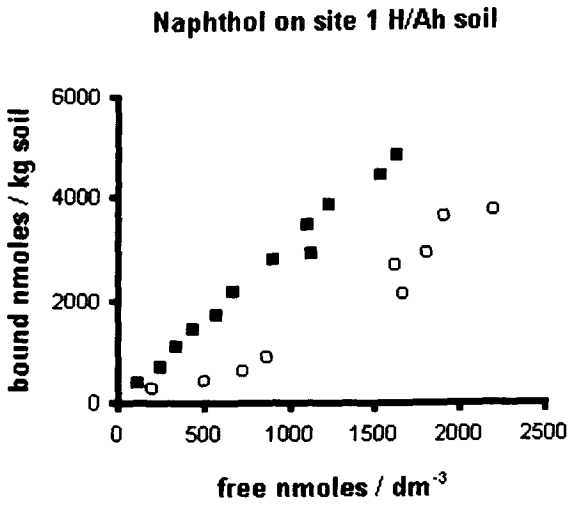


Figure B11

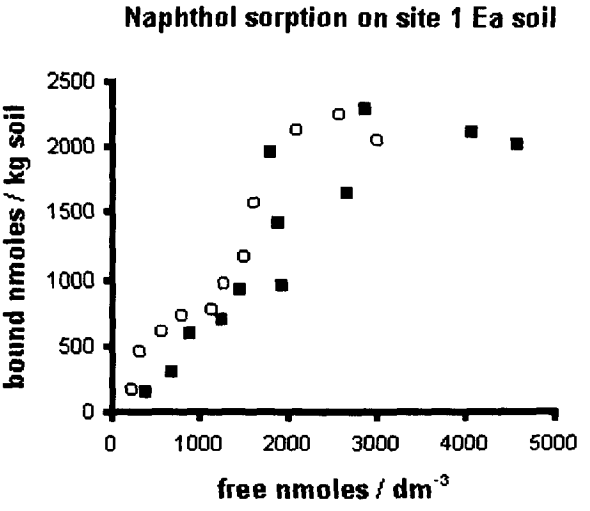


Figure B12

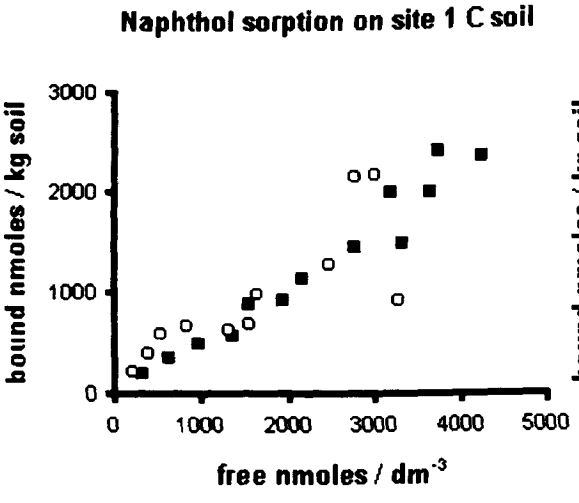


Figure B13

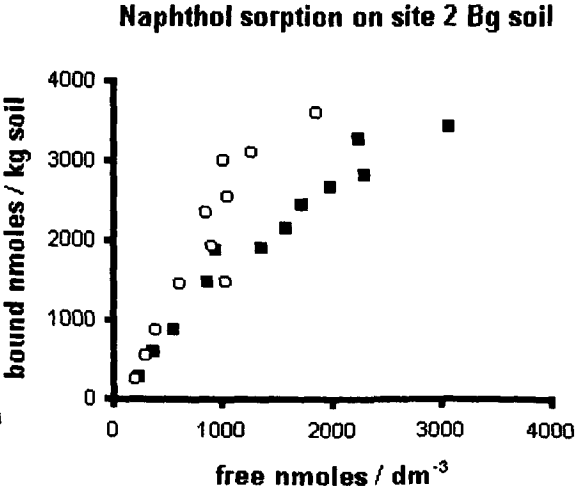


Figure B14

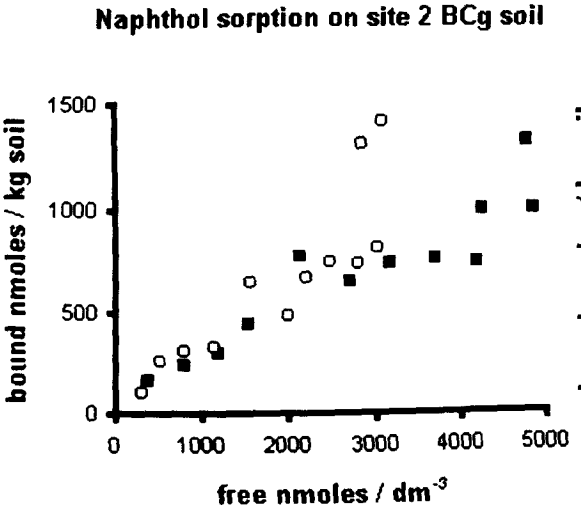


Figure B15

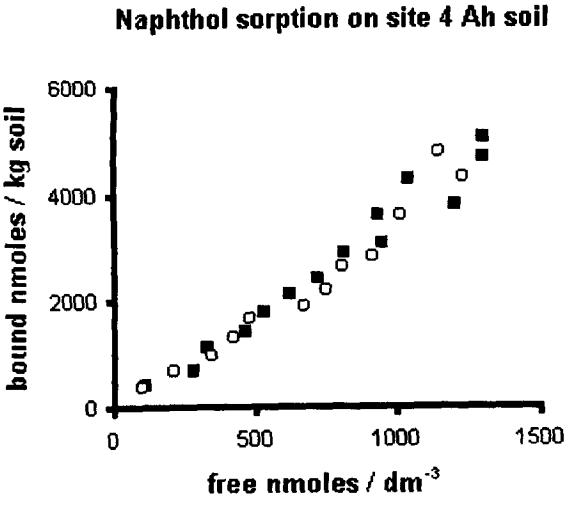


Figure B16